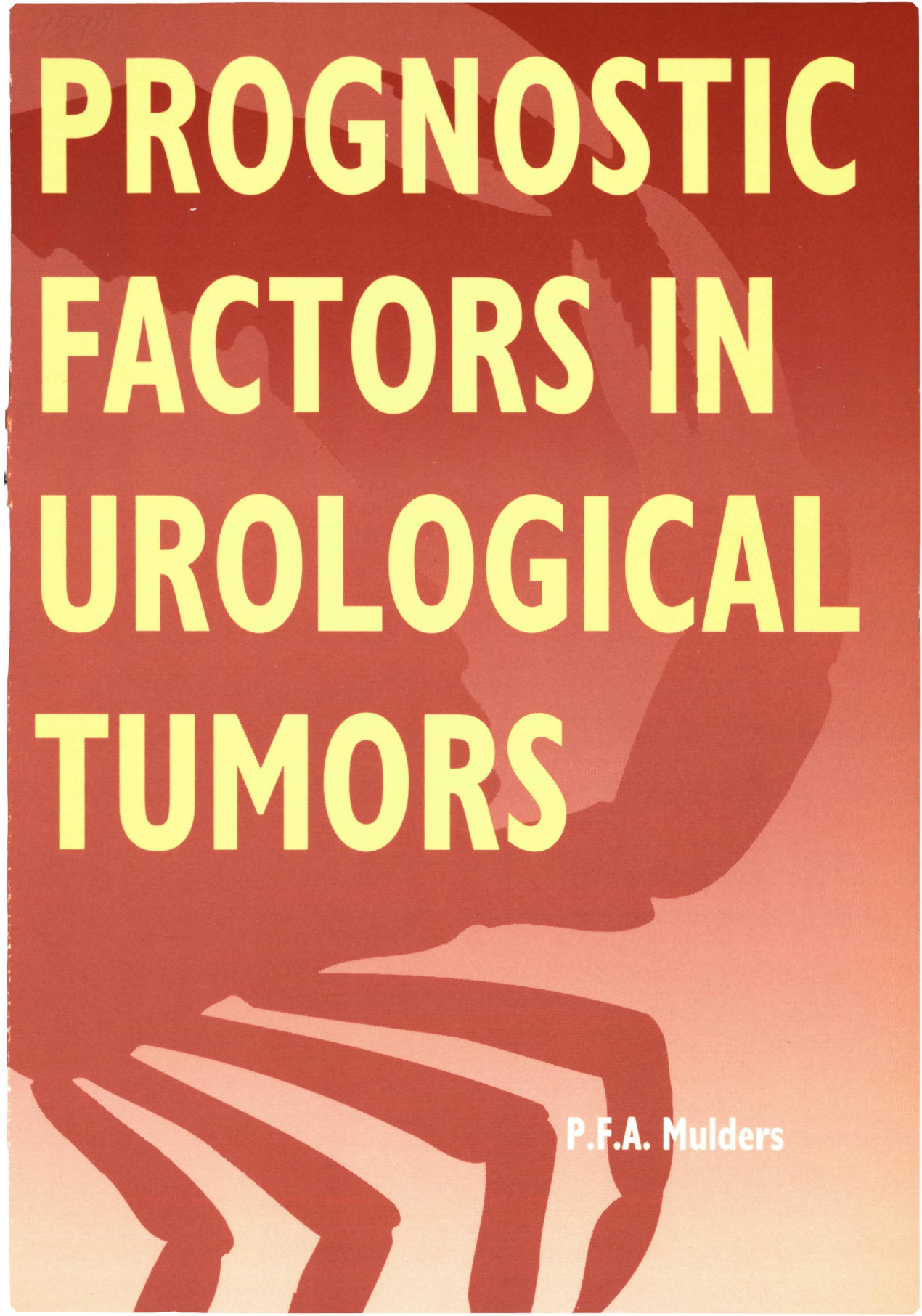
PROGNOSTIC FACTORS IN UROLOGICAL TUMORS



P.F.A. Mulders

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PROGNOSTIC FACTORS IN UROLOGICAL TUMORS

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen,
in het bijzonder de Geneeskunde

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volgens besluit van het college van decanen
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Promotor: Prof. Dr. F.M.J. Debruyne

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"I appears to me a most excellent thing for the physician to cultivate ***Prognosis****; for the foreseeing and foretelling, in the presence of the sick, the present, the past, and the future, and explaining the omissions which patients have been guilty of, he will be the more readily believed to be acquainted with the circumstances of the sick; so that men will have confidence to intrust themselves to such a physician."*
In: The Genuine works of ***Hippocrates****, the Book of Prognostics, p42, translated by F.A. Adams, 1972*

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CONTENTS page

CHAPTER I

GENERAL INTRODUCTION

1. Prognostic factors in urological oncology

During the last decades many efforts have been made to define prognostic factors in oncological urology, attempting to keep pace with the individual need of new therapeutic options for patients with cancer. Prognostic parameters related to the patient and his cancer may influence the choice of treatment. With these prognostic factors, subsets of patients can be defined that correspond to the characteristics of their disease.

Questions as to the risk for recurrence or the chance for survival, are of importance in daily clinical practice. Each physician must be aware of the natural history of the tumor and its response to treatment, in order to be able to inform the patient. We are still unable to predict the exact course of the disease for the individual patient by using prognostic factor techniques. We have learned from our statisticians that we can only make statements about the probability of recurrence and survival. With these restrictions in mind, research on prognostic factors remains of great importance for the analysis in clinical oncological studies.

First of all, we have to clarify the definitions of prognosis and of a prognostic factor in order to be able to use these terms (Fletcher, 1988). Prognosis is a prediction of the future course of the disease, following its onset. Prognosis depends on the response of the patient to the cancer, the potentiality of the neoplasm, and the kind of treatment received. Prognostic factors are the conditions which, when present in persons already known to have the disease, are associated with the outcome of the disease. Prognostic factors can be subdivided by a natural classification which includes (1) host factors, (2) tumor characteristics, and (3) the effects of the tumor on the host (Byar, 1986). Examples of the first category are age, sex and the blood group. Examples of the second are the histological and morphometric characteristics, while performance status, laboratory results and metastasis patterns are examples of the last category.

Knowledge of prognostic factors may assist in the design of future trials in several ways. Prognostic factor studies permit us to focus on therapeutic regimens in appropriate subsets of patients and should lead to an improvement in the basic understanding of tumor biology. For some planned treatment comparisons, it may be desirable to stratify the patients according to prognostic groups. In this way, patients can be divided into good, intermediate and poor risk groups according to certain prognostic factors. Risk

group membership can be used as an indication of the prognostic variables for each member, and may provide information for the individual patient. It will also make these patients accessible for treatment comparison and can therefore be used for a stratified randomization in future studies. A 'prognostic stratification' should be at the basis of most choices of therapy.

Mainly because the clinician is interested in answers about the probable course of the disease, they are interested in the results of studies of prognostic factors. A clinician arrives at a prognosis for a new patient by means of recalling the results in a group of previously treated patients with the same characteristics. However, this resemblance is merely an incomplete abstraction of the results from prognostic analyses. Prognostic factor recognition is an important step towards the notion that treatment in oncological patients must have an individualized basis, mainly because of the heterogeneity of the characteristics of the patient and his tumor.

New treatment modalities may improve the survival of patients. Almost every new trial in which certain subsets of patients with cancer are treated, is nowadays accompanied by an analysis of its prognostic factors. The results will help in the evaluation of the trial and will be beneficial for the development of future studies.

For the urologist, patients with cancer constitute a substantial part of his practice. Also for this purpose, it is essential to develop a classification of cancer based upon anatomic and histological considerations. This is the cornerstone of cancer decision-making as a multidisciplinary process. Anatomical staging gives information about the extent of the disease. This is done by the Tumor, Nodes and Metastases (TNM) classification. The International Union Against Cancer (UICC) is one of the most important organisms in the field of the classification of malignancies (UICC, 1987). Its objectives will help the clinician to plan treatment, assist in the evaluation of the treatment results for clinical research, and facilitate the exchange of information.

Statistical considerations

The role of the statistician must be clearly defined. The statistical groundwork of the prognostic factor analysis in clinical oncology must be performed by professional statisticians. An independent specialist in this field should, in principle, guarantee the objectivity of the outcome of our clinical studies. Physicians must know and be able to

interpret these results and use them to inform their patients.

The results of a prognostic factor analysis must be interpreted with caution, because they depend on several constraints by which every study is influenced. Usually a prognostic factor analysis is performed in a retrospective way. The results will be influenced by the disadvantages of this method. For example, missing data and drop-outs for unknown reasons will influence especially the results of a multivariate analysis. Missing data require a listwise non-random selection of patients for statistical analysis with possibly biased results. However, advantages of a retrospective study are that the results can be obtained within a relatively short period of time. These results may than be helpful in designing future studies.

Prognostic factor analysis can also be performed as a side-study of the cancer treatment protocols. These trials have the advantage of a good documentation. The results from the prognostic factor analysis of these studies are a function of the study design. First of all, the results of the prognostic factor analysis may be influenced by the accrual periods of patients. In this way different groups of patients with the same tumor characteristics, but recruited at different times, may possess different prognostic values. For example the inclusion of patients in a study may depend on the current opinion of the physician on a particular therapy. The results will also be influenced by the inclusion criteria of the study. It is important to take into account all the inclusion criteria before drawing any conclusion. Another important feature is the number of characteristics which will be included in the prognostic factor analysis. The prognostic value of a certain characteristic, may be different when it is included in another set of factors. The number of treatment arms of the trial is also of importance. When the prognostic factor analysis is restricted to treatment, still slight differences may have a great impact on the outcome of the prognostic analysis. The number of physicians and centres (in case of a multicenter study) from which the patients are recruited is also important for the study design. Unless an adequate protocol design exists, differences between different centres may occur and will influence the outcome of the results. From all these statements, it must be concluded that it is hazardous to group the results of different studies for one prognostic factor analysis. The advantage of a large number of patients decreases by the diminishing reproducibility of the results. From a statistical point of view it is better to have results from an independent prognostic factor analysis.

The results of the prognostic factor analysis will also be influenced by the technical-statistical procedures. The choice of statistical tests and the development of statistical analytic models (proportional hazard model versus logrank or Wilcoxon model free) will influence the results (Cox, 1972). The variables may be analyzed simultaneously to detect the functional relation of each factor. Some relation between prognostic factors is always present, and must be taken into account in order to arrive at valid conclusions. An explanation of more technical aspects is however beyond the scope of this thesis. Certain standard procedures are available to diminish the subjectivity of choice.

In this chapter, we want to summarize the most relevant prognostic factors in the four most prevalent urological tumors, namely the renal cell carcinoma, superficial bladder tumors, disseminated prostatic carcinoma and disseminated germ-cell tumors. This will be the basis for our investigations which will be presented in the following chapters of this thesis.

2. Prognostic factors in germ-cell tumors

2.1. Introduction

In contrast with the other urological neoplasms the prognosis of germ-cell tumors is good, even in an advanced stage. The survival for the several stages ranges from 70 to 95% (Zwaveling, 1985). The improvement in survival is mainly due to the development of cis-platinum based combination chemotherapy in 1976 for disseminated germ-cell tumors (Einhorn, 1977). However, this progress should not lead to resignation in the management of these patients. Several studies have been done to get a better insight in the timing and extend of the different phases of treatment (Donohue, 1979). As a result, it is now generally accepted that the treatment strategy of advanced stages of disseminated germ-cell tumors should be primary chemotherapy and not primary debulking surgery (Donohue, 1980, Einhorn, 1981, Donohue, 1984, Stoter, 1984).

The good prognosis of the different stages of the disease has changed the interest of treatment towards the reduction of side-effects from both chemotherapy and surgery without affecting the good survival rate. On the basis of prognostic factors patients are divided in different risk groups (good, intermediate, and poor prognosis) and the treatment can be adapted accordingly. The individualization of treatment might thus lead to less cycles or a lower dose of chemotherapy or a less agressive or even abandoned surgery for patients with good prognostic factors and more agressive therapy for those at high risk (Einhorn, 1987, Williams, 1987, Donohue, 1988, Lotherington Qvist, 1991). It is important, however, for trial comparison afterwards, to use uniform entry criteria (Bajorin, 1988, Bosl, 1991). Studies on prognostic factors are thus important in order to establish the least aggressive treatment in patients with a good prognosis and to improve survival in patients at risk.

In this chapter we want to summarize the most relevant prognostic factors known in literature and used for treatment decisions.

2.2 Pathologic prognostic parameters

The histology of the primary germ-cell tumor obtained after orchiectomy has been used as a classification with prognostic implications (Dixon, 1953). Nowadays the two mostly applied classifications are the British by Collins and Pugh and the WHO by

Mostofi and Sobin (Collins, 1976, Mostofi, 1977). Table 1 shows these two classification models.

Table 1: Classifications of histology in germ-cell tumors

Collins and Pugh (1976)	Mostofi and Sobin (1977)
Seminoma Spermatocystic	Seminoma Spermatocystic
Teratoma, differentiated (TD)	Teratoma Mature Immature With malignant transformation
Malignant teratoma, intermediate (MTI)	Embryonal carcinoma and teratoma
Malignant teratoma, undifferentiate (MTU)	Embryonal carcinoma (adult type)
Malignant teratoma, trophoblastic (MTT)	Choriocarcinoma with or without embryonal carcinoma and/or teratoma
Yolk sac tumor	Yolk sac tumor (endodermal sinus tumor)

Modifications on these classifications have been proposed but were not included mainly because of the risk of confusion and the need for comparable groups in international literature (Mostofi, 1990). The most important consequence for therapy which is derived from these histologic classification models is the distinction between semonima and non-seminoma. Treatment for early stages differs substantially. In the advanced stage all germ-cell tumors are treated by chemotherapy in the first place, as previously mentioned.

Within these classification models there are some histologic patterns which have prognostic importance. Vascular and/or lymphatic invasion, the presence of yolk-sac elements, and the presence of undifferentiated tumor, independently were related to a higher relapse rate, especially, stage I non-seminomatous germ-cell tumors (Javadpour, 1986, Freedman, 1987, McLeod, 1991). Patients with metastases of a primary tumor containing teratomatous elements have a worse prognosis and this may have implications for treatment decisions (Javadpour, 1986, Donohue, 1987, Lotherington Qvist, 1991).

2.3 Radiographic prognostic parameters

After orchiectomy accurate identification of the extent of the disease in the individual patient is mandatory for an appropriate prognostic classification and consequently for the choice of treatment. The generally accepted staging system is the Royal Marsden classification (Peckham, 1979) (Table 2).

Table 2 : Royal Marsden Classification

STAGE	I: Tumor limited to the testis.
STAGE	II: Infradiaphragmatic nodal disease

(The diameters relate to cross-sectional measurements, not vertical.)

	IIA: Retroperitoneal metastases < 2 cm diameter
	IIB: „ „ 2-5 cm „
	IIC: „ „ 5-10 cm „
	IID: „ „ >10 cm „
STAGE	III: Supradiaphragmatic nodal disease
STAGE	IV: Haematogenic metastases (liver, lung, brain, etc)

Improvements of imaging technics, especially CT-scanning, have been made and increased the accuracy of this staging of especially retroperitoneal lymphnode metastases (Husband, 1985, Kennedy, 1985). The accuracy of these imaging techniques is however not higher than 70%. The evaluation of retroperitoneal mass before and after chemotherapy is also essential in the decision of adjuvant surgical treatment (Donohue, 1982, Oliver, 1983, Stomper, 1985, Donohue, 1987). CT-scanning is usefull in this prospect because of its correlation with postchemotherapeutic histologic surgical outcome (Husband, 1982, Stomper, 1985). The usefulness of the magnetic resonance impedance (MRI) concerning adequate detection of primary tumor location and the follow-up, still has to be established.

2.4 Biochemical prognostic parameters

The identification of the tumormarkers alpha-fetoprotein (α-FP), beta-subunit human chorionic gonadotropin (ß-HCG) and lactic dehydrogenase (LDH) are useful in the determination of prognosis in germinal tumors (Lange, 1977, Scardino, 1977,

Newlands, 1983, Droz, 1988). They are used to establish the diagnosis, to monitor response to chemotherapy and to detect recurrent disease (Lange, 1977). The presence in the serum of these tumormarkers is related to the histological pattern of the tumor and therefore have a prognostic impact. Quantitative measurements also show a correlation with survival. Therefore these tumormarkers, wether or not in combination with other features, can be used in the determination of risk groups and the development of classification models (MRCWP on Testicular Tumours, 1985, Birch, 1986, Bajorin, 1988, Droz, 1988, Bosl, 1991).

Table 3 contains a summery of investigations on prognostic factors in disseminated germ-cell tumors.

Table 3: + = significant correlation, - = no significant correlation

	Bosl '83 n=171	MRCWP '85 n=458	Birch '86 n=180	Stoter '87 n=163	Droz '88 n=84	Mead '92 n=795
trophoblastic components	-	+	-	+	-	+
ß-HCG	+	+	+	+	+	+
α-FP	-	+	+	+	+	+
LDH	+			+		
tumor size		+		+		
abdominal metastases	+	+	+	+	+	+
lung metastases		+	+	+	-	+
liver,brain,bone metastases		+	+			+

It is essential in the management of germ-cell tumors to use a combination of histological, radiographic and biochemical parameters in order to make a treatment decision. Treatment often includes chemotherapy and surgery and this intensive therapy has attributed to the good prognosis of this tumor. Recently classification models of prognostic factors have been developed. The European Organization for Research and Treatment of Cancer (EORTC) classification of prognostic factors and the Indiana

classification of extend of disease attempt to categorise patients in order to predict the results of treatment (Birch, 1986, Stoter, 1987). In both cases risk groups are defined and the treatment can be adapted accordingly. These models are shown in table 4 and 5.

Table 4: EORTC CLASSIFICATION MODEL

Good prognosis (all of the following)
- Lymph node metastases < 5cm in diameter
- Lung metastases < 4 in number, < 3 cm in diameter
- beta-HCG < 1000 ng/ml (< 5000 IU/l)
- alpha-FP < 1000 ng/ml

Intermediate prognosis (any of the following)
- Lymph node metastases 5-10 cm in diameter
- Lung metastases > 4 in number
- Lung metastases > 3 cm in diameter
- beta-HCG < 1000-9999 ng/ml (5000-49999 IU/l)
- alpha-FP > 1000ng/ml

Poor prognosis (any of the following)
- Lymph node metastases > 10 cm in diameter
- beta-HCG > 10000 ng/ml (>50000 IU/l)
- Extragonadal germ cell tumors
- Metastatic sites other than lymph nodes and lung (liver, bone, brain, etc)

Table 5. Indiana classification model

Minimal
1 Elevated beta-HCG and/or alpha-FP only
2 Cervical nodes (non-palpable retroperitoneal nodes)
3 Unresectable, but nonpalpable, retroperitoneal disease)
4 Minimal pulmonary metastases--less than five per lung field and the largest <2 cm (non-palpable abdominal disease)

Moderate
5 Palpable abdominal mass as only anatomical disease
6 Moderate pulmonary metastases--five to ten pulmonary metastases per lung field and the largest <3cm or a mediastinal mass <50% of the intrathoracic diameter or a solitary pulmonary metastasis any size >2cm (nonpalpable abdominal disease)

Advanced
7 Advanced pulmonary metastases--mediastinal mass >50% of the intrathoracic diameter of greater than ten pulmonary metastases per lung field or multiple pulmonary metastases >3cm (nonpalpable abdominal disease)
8 Palpable abdominal mass plus pulmonary metastases
8.1 --minimal pulmonary
8 2 --moderate pulmonary
8.3 --advanced pulmonary
9 Hepatic, osseous, or CNS metastases

In chapter II we performed an analysis of our patients with disseminated germ-cell tumors based on these classification models. We also looked for additional parameters that may be of use to predict the histological outcome of residual tumor after chemotherapy.

3. Prognostic factors in prostate cancer

Based on: Predicting treatment response in metastatic prostate cancer
P.F.A. Mulders, F.M.J. Debruyne, G.O.N. Oosterhof
Reviews on Endocrine-Related Cancer (1991), 38, 5.

3.1 Introduction

Prostate cancer is one of the major causes of death in older men in western countries. Epidemiological studies have shown that there is still an increase in the incidence of prostate cancer (Silverberg, 1989). In about 60-70% of the patients prostate cancer is diagnosed in an advanced stage (Catalona, 1986). Two major points of interest in the management of prostate cancer are the detection of the tumor in an early curable stage, when the disease is still localized, and the development of new treatment modalities for disseminated disease.

Since more than 45 years, disseminated prostate cancer is treated with hormonal therapy (which is still the treatment of choice) (Huggins, 1941). However, at least 20-25% of the patients with metastatic disease do not react on hormonal treatment, and an equal percentage becomes resistant to the treatment within 2 years (Resnick, 1978). New therapeutic approaches are needed to prevent growth of both hormone-dependent and hormone-independent tumor cells from the onset, delarging time to progression and therefore improving survival (Grayhack, 1987). The results of treatment with combined hormonal and cytotoxic drugs were reviewed by several authors, but none of these have shown a substantial advantage, mainly because of limiting side-effects in these older patients (Gibbons, 1987). New therapeutic strategies are especially needed for the patients who are refractory to standard treatment.

During the last years an increasing number of treatment modalities were developed for patients with disseminated prostate cancer. It was observed that the response to treatment and thus survival varies considerably in this group of patients. In order to be able to identify, before the start of treatment, the patients with a poor prognosis, it is useful to analyze the factors that influence survival.

More knowledge on prognostic factors is also essential for the design of future

studies. They may give objective information on expected response to treatment and therefore on survival. Also the use of parameters which can monitor these new treatment modalities are essential and must be clarified with regard to the objective response of prostate cancer.

This chapter aims to give an overview of clinical, pathological, radiographic and biochemical prognostic parameters and discuss their importance in predicting response directly related to progression and survival in patients with disseminated prostate cancer.

3.2 Clinical prognostic parameters

Age has been mentioned as a prognostic indicator (Berry, 1979, Wilson, 1984). It was concluded that, generally, younger patients had a more aggressive tumor, and therefore a worse prognosis than elderly patients. However, this is could not be confirmed by the results of others (Harrison, 1983, Emrich, 1985, Soloway, 1990, Ernst, 1991).

Another clinical parameter is the performance status, mostly categorized by the Karnofsky classification. De Voogt and associates showed this to be the most important prognostic indicator, and an increasing number of investigators confirmed this finding (de Voogt, 1989). A bad performance status is mostly associated with a shorter survival (Emrich, 1985, de Voogt, 1989, Soloway, 1990). This parameter is related with the advanced stage of the disease and it is useful in daily practice when treating patients with disseminated prostate cancer. Performance status as a factor in prognosis may be of more importance when used in combination with the pain score (Emrich, 1985).

3.3 Pathological prognostic parameters

As for pathological parameters, grade has been most frequently used to correlate histologic characteristics with biological activity. There are two widely used classifications, the Gleason and the Mostofi system. Both have been correlated with prognosis in prostate cancer (Gleason, 1974, Mostofi, 1975). During the last decades these classifications have been reconsidered, but they all share limited efficacy with regard to predicting response to treatment in the individual patient. Some studies show prognostic significance especially for the poorly differentiated tumors in all stages of prostate cancer (El-Mahdi, 1983, Grayhack, 1983). De Voogt and Ernst indicated the positive prognostic

value of grade on survival (de Voogt, 1989, Ernst, 1991)

Other types of histological parameters were looked for in order to predict response to treatment. First there are several studies on morphometrical aspects. The nuclear roundness factor initially showed interesting perspectives with regard to biological aggressiveness of the tumor (Diamond, 1982, Epstein, 1984). This potential prognostic parameter has not been used widely because the determination is time consuming and a significant variability is seen, related to tissue handling and preparation. On the other hand we believe that in the near future this parameter will be used again in more simplified and automated techniques and its use for the clinician will be re-established.

Another prognostic approach is flowcytometry. Although much research has been done in this field, the results for prostate cancer are less promising than in other tumors (Schultz, 1985). Tannenbaum et al. found that the prognosis of these patients was related with ploidy (Tannenbaum, 1982).

Recently a new prognostic parameter was described by Cohen and associates, who found a better survival in the patients without neuro-endocrine staining cells in their specimen (Cohen, 1990). This indicates that probably more results, derived from histologic and immunohistochemical examinations will follow and help us to individualize treatment in patients with prostate cancer.

3.4 Radiographic prognostic parameters

Bone is the only site of distant metastases in 65% of patients who present with disseminated prostate cancer (McCrea, 1988). Soloway et al. proposed a scheme to grade the extent of the disease observed on bonescan (Soloway, 1988). This classification opened the possibility to evaluate the number of bone metastases during hormonal treatment. Ernst et al. found that the extent of the disease on bonescan was the most important prognostic factor in their analysis (Ernst, 1991). Cooper and Soloway et al. found that the survival was worse with an increasing number of metastases on bone scintigraphy and proposed that this parameter should be used for prognosis (Cooper, 1990, Soloway, 1990).

3.5 Biochemical prognostic parameters

Parameters determined from laboratory results have been examined extensively

during the last decades. Also because of economic reasons the establishment of these parameters is of great importance. In the majority of the investigations laboratory parameters have been compared with objective response on bonescan (Donoghue, 1978).

The prognostic significance of alkaline phosphatase in patients with metastatic disease has been established, but is still underestimated (Wilson,1985). Prostate-acid-phosphatase (PAP), is generally accepted to be of prognostic significance for survival (Babaian, 1986). However, more recent studies have shown that this parameter is of less value in disseminated prostate cancer, especially when compared to alkaline phosphatase and prostate specific antigen (PSA) (Killian, 1986, Haapiainen, 1990). This last tumor marker, first described by Kuriyama and Wang in 1981 (Wang, 1981), has become one of the most investigated biochemical tests for prognosis. PSA can be used for early detection of the disease, to establish time to progression and for monitoring the effects of treatment. PSA is not an appropriate marker in the sreening of prostate cancer due to its lack of sensitivity (Wang, 1981, Guinan, 1987, Cooner, 1988). The role of PSA as a pretreatment prognostic parameter has not yet been established. In the original report of Kuriyama et al. a high level of PSA seemed to be associated with a poor survival, but these results were not significant (Kuriyama, 1981). PSA is, however, an extremely valuable marker in monitoring the tumor during treatment. Guinan et al have established the significant value of PSA in monitoring patients with prostate cancer (Guinan, 1987). Cooper et al. showed that prognosis is good in patients with a very low PSA (< 10 ng/ml) six month after the start of hormonal treatment, but he used this factor in a combination with bone scanning (< 15 lesions) to obtain significant results (Cooper, 1990).

Other laboratory parameters than the above mentioned are less investigated. Soloway recently described the value of pretreatment testosterone level (Soloway, 1990). A low testosterone level before the start of treatment was associated with a shorter survival. In a multivariate analysis he used this factor together with the performance status and number of bone lesions to obtain significant prognostic groups (Soloway, 1990). Also other investigations showed that patients with low pretreatment testosterone levels were less responsive to androgen deprivation (Harper, 1984, Ernst, 1991).

In table 6 we summarize the most frequently used prognostic parameters in disseminated prostatic cancer.

Table 6: + = significant correlation, - = no significant correlation

	Berry '79 n=88	Emrich '85 n=1020	de Voogt '89 n=436	Soloway '90 n=110	Ernst '91 n=162
age	+	-	+/-	-	-
performance status	+	+	+	+	-
Hb		+	+		
PSA					-
PAP	+	+	+	-	-
AlkP	+	+	+		
testosterone				+	+
grade		-	+		-
number of bone metastases				+	+

Patients with disseminated prostate cancer who fail the initial hormonal treatment have a mean survival of less than one year (Catalona, 1986). There are several hypotheses

for this failure to response to hormonal treatment. The presence of heterogeneous clones of carcinoma cells is mostly accepted as being the cause of this phenomenon (Isaacs, 1981). From the onset of the malignancy there must be hormone-dependent and hormone-independent cells in the tumor. During the initial hormonal treatment, hormone-independent cells continue to proliferate and later become evident as tumor progression. Another hypothesis is the change of the individual cells in their genetic requirements of androgen for growth, which can result in hormonal resistance (Isaacs, 1981)

Because of the low efficacy and considerable side-effects there is still limited place for chemotherapy in the treatment of hormone resistant prostate cancer (Tannock, 1985). This is also the reason why much research has been done on "salvage" endocrine manipulation. Despite the number of investigations in this field, it can be concluded that no evidence for prolonged survival could be established. The only rationale for using second-line hormonal treatment is the subjective improvement in 20 to 30%. (Narayama,

1981, Williams, 1986). It is difficult to predict which patient will react on salvage endocrine treatment. Serum testosterone levels may indicate the failure of a primary hormonal treatment, and the patient may have benefit from a bilateral orchiectomy. Geller et al. showed the usefulness of determining the DHT concentration in prostate tissue (if greater than 2.4 mg/gm), because it can indicate persistent androgen production which may react on secondary hormonal manipulation (Geller, 1984). The most important problem of predicting reaction on secondary hormonal manipulation is the lack of follow-up, due to the limited duration of response.

In conclusion we are convinced that treating patients with disseminated prostate cancer with standard therapy is not in accordance with the heterogeneity of the tumor. Factors predicting response to treatment can be determined from clinical, pathological, radiographic and laboratory parameters. Further studies of the alreadey known parameters and the development of new parameters are necessary. For this reason we performed side studies of trials on hormonal treatment for disseminated prostate cancer which were organized by the Dutch South Eastern Oncology Group. These trials are well monitored and therefore accessible for prognostic factor analysis. The results are shown in chapter III and IV.

4. Prognostic factors in bladder cancer

4.1 Introduction

Bladder cancer is one of the most prevalent cancers in male, especially because of the high recurrence rate (Silverberg, 1987). Tumors of the bladder are mainly divided into superficial and muscle-invasive cancers, each with its own clinical consequences. Approximately 85% of bladder cancers present as superficial tumors (American Cancer Society, 1987). Superficial bladder tumors are considered as a homogeneous group with consequences for the treatment and the follow-up shedule.

Because of the high incidence most studies concentrate on superficial bladder cancer, in an attempt to decrease the recurrence rate and to prevent progression into muscle-invasive disease. One of the most important features of the superficial bladder tumor is the high incidence of recurrence following initial transurethral tumor resection. Recurrence rate ranges from 50 to 80% (Lutzeyer, 1982). This is because the disease is considered to affect the whole urethelium. Every new, in onset superficial tumor, may progress into an invasive cancer. This risk is estimated to be 25-30% (Heney, 1983). This means that the follow-up of a patient with superficial bladder cancer is a continuous process and that the patient remains under surveillance during the rest of his life.

The high tendency to recur and the risk of progression into invasive disease is the ratio for studies on new treatment possibilities, especially new intravesical therapies. Many prospective randomised studies have been performed since the first report on the beneficial effects of intravesical chemotherapy by Jones and Swinney and Veenema (Jones, 1961, Veenema, 1969). The importance of intravesical chemotherapy and later of intravesical immunotherapy is now established. In the past it was mainly used after inclompete resection of a tumor and in case of carcinoma in situ (Morales, 1981, Edsmyr, 1984, Jakse, 1984). During the last years more attention was given to the prophylactic use of intravesical chemotherapy and especially immunotherapy (Morales, 1978, Koontz, 1981, Lamm, 1985, Herr, 1986). Today there is consensus about the beneficial effect of this treatment on superficial bladder tumors and carcinoma in situ with regard to the decrease of recurrence rates (Herr, 1987, Kurth, 1989). On the other hand it appeared that not all the superficial bladder tumors react the same on this treatment (Herr, 1991). Some superficial bladder tumors with a high tendency for recurrent and progressive

disease did benefit more from the adjuvant treatment. On the other hand for some other less aggressive tumors intravesical therapy appeared to be of no use. This is why individualization of treatment is mandatory (Rubben, 1988).

Considering the new developments in the treatment of superficial bladder cancer, the research on prognostic factors is essential. Prognostic factor analysis may lead to the formation of certain risk groups with consequences for the choice of treatment (Herr, 1989). Within this chapter we want to summarize the prognostic factors which are of interest for superficial bladder tumors.

4.2 Clinical prognostic parameters

Possible clinical factors like age and sex have been determined, but no clear prognostic value has been found (Pocock, 1982, Narayama, 1983, Herr, 1989).

The patient with a superficial bladder cancer is mostly not suffering physically from the tumor; this means that it is not relevant to examine features like weight-loss, performance status and pain.

In bladder cancer, a radiographic characteristic like the dilatation of the ureter on intravenous urography may indicate muscle invasive disease and therefore a worse prognosis (Hatch, 1986, Golding, 1987).

One of the most important independent prognostic factors for recurrent disease is the multiplicity of the tumor. In several studies this appeared to be of major importance (Lutzeyer, 1982, Dalesio, 1983, Parmer, 1989, Kurth, 1989). This characteristic is already included in the design of several new trials. The size of the largest tumor as a prognostic factor is controversial. In contrary with Lutzeyer et al. and Kurth et al., who found no prognostic impact, several other investigations included this factor as a positive prognostic sign (Lutzeyer, 1982, Heney, 1982, Dalesio, 1983, Kurth, 1989, Parmer, 1989).

4.3 Pathological prognostic parameters

The importance of urine cytology in the detection and follow-up in patients with bladder cancer has been established (Lewis, 1976, Murphy, 1984, Koss, 1985, Meuleman, 1988). Especially the occurrence of the combination of a positive cytology and a high grade bladder tumor is striking (Koss, 1985, Meuleman, 1988). Supplementary tests with

cytology like transferrin receptor expression and quantitative immunocytology show adequate predictive value on risk for recurrence (Smith, 1990, Huland, 1990).

One of the most important classification models derived from histologic features is the TMN classification (IUCC, 1987). The ultimate goal of this system was to standardize the subdivision of patients with bladder cancer and consequently make them accessible for treatment comparison (Prout, 1977). Retrospective analysis on subdivisions of patients could be performed (Cifuentes Delatte, 1982). An important modification for superficial pT_1 tumors was developed by the seperation pT_a and PT_1 (Chisholm, 1980). The significance of lamina propria invasion (pT_1) on prognosis was indicated (Anderstrom, 1980). It was questioned whether pT_1 tumors should still be classified as "superficial" and if they had to be treated with another strategy (Heney, 1982). Today tumor stage is included in every prognostic analysis of superficial bladder tumors. Especially with regard to the natural history of bladder tumors, tumor-stage appeared to be of significant prognostic value (Heney, 1982, Lutzeyer, 1982). However, when treated with intravesical instillations of especially BCG, tumor stage distinction between pT_a and pT_1 showed to be of less prognostic significance (Shinka, 1990). These instillations can alter the biological behaviour of high risk tumors and influence the prognosis. Torrence et al. did not find a significant influence of tumor stage on recurrence-free interval (Torrence, 1988).

Grade is a histological feature which has shown its usefulness in the management of superficial bladder tumors. Its application on bladder tumors was already performed in 1922 (Broders, 1922). Histological typing of bladder tumors has been introduced as a classification system by Mostofi (Mostofi, 1973). A clear relation between grade and prognosis was shown (Gilbert, 1978). Malkowics et al., after evaluation of the influence of the grade on prognosis, advised to treat high grade superficial bladder cancers more aggressively with radical cystectomy (Malkowics, 1990). However, restrictions of the value of grade must be made because different interpretations of grading by different pathologists became obvious, and this inter-individual variation will have consequences in clinical decision making (Ooms, 1983). Therefore, objective criteria derived from quantitive histological features are of importance. Attempts have been made to increase the usefulness of histologic grading as a prognostic factor in patients with bladder carcinoma (Pagano, 1987, Pauwels, 1988).

A combination of stage and grade appeared to be of importance for prognosis (Kern, 1984). Especially the pT_1G_3 tumors are at risk for recurrent disease and this unfavourable outcome is discussed in several studies (England, 1981, Jakse, 1987, Abel, 1988, Birch, 1989, Jenkins, 1989).

Another characteristic which is possibly associated with prognosis is the histology of the associated random biopsies. The results of biopsy of normal-looking mucosa appeared in several studies to be of influence on recurrence (Loening, 1978, Soloway, 1978, Smith, 1983, Wolf, 1983). It is an indication that bladder cancer affects the total urothelial surface. This also explains why, especially patients with carcinoma in situ, require adjuvant treatment like intravesical chemotherapy or immunotherapy to decrease the risk for recurrent disease (Herr, 1983, Quilty, 1987).

Table 7 summarizes the most important clinical and histological prognostic factors that are of interest in the treatment of superficial bladder cancer.

Table 7: + = significant correlation, - = no significant correlation

	Lutzeyer '82 n=315	Dalesio '83 n=308	Heney '82 n=249	Parmer '89 n=379	Kurth '89 n=371
History of recurrence	+	+			+
Multiplicity	+	+	+	+	+
size of tumor		+	+	+	-
T-stage	+	(only T_1)	+	-	+
Grade	+	+	+	+	+

4.4 Biochemical and cytogenic prognostic parameters

Because superficial bladder tumors form a heterogeneous group in their clinical course, studies on adjuvant prognostic factors remain essential. Until now no additional prognostic value is derived from laboratory results which can be used in daily clinical practice. The usefulness of features like blood-group antigens, specific red cell adherence, receptor status, T-antigens and other monoclonal antibodies have shown prognostic significance, but so far no widespread clinical application has been achieved (Stein, 1981, Fradet, 1986, Srivinas, 1986, Blasco, 1988). It is, however, essential to continue the search for factors that can be of use for the routine evaluation of patients with bladder tumors.

In the last decade flowcytometry has been developed as an objective method for determination of the recurrence-free interval. Several studies describe that additional information for the prognosis may be derived from flowcytometry (Hadjissotiriou, 1985, Masters, 1986, Blomjous, 1988). Murphy et al. underlined that the routine use of the flowcytometric technique remains controversial, because no strong additional prognostic potential could be found, as compared to routine cytological and histological grading (Murphy, 1986).

Another factor which has been evaluated is morphometry. Recently, studies have shown that morphometric criteria are superior to flowcytometry, as an additional feature for patients with superficial bladder tumors (Ooms, 1981, Lipponen, 1990). Routine use of this time-consuming technique is not yet appropriate. The role of abnormal chromosomal markers with regard to prognosis of superficial bladder cancer has been subject of investigation (Sandberg, 1977, Falor, 1988). Although the results are promising, the usefullnes for predicting prognosis in clinical practice remains uncertain.

Patients with superficial bladder cancer are characterized by their heterogeneous clinical course and unpredictable reaction on treatment. Prognostic factor analyses are useful to individualize treatment and to achieve the best possible outcome for these patients. Superficial bladder tumors are not aggressive in their neoplastic behaviour and research is concentrated on the high recurrence rate in some of these tumors. The consensus on the positive effect of intravesical immuno-, and chemotherapy after initial transurethral resection of all visible tumor has changed the approach of bladder tumors considerably. When different treatment options exist, there is a need for an adequate indication to choose the right treatment schedule. Prognostic factor analysis show different results in patients treated with or without adjuvant therapy. Review articles and consensus meetings are important in order to define standard treatment and follow-up shedules and to initiate the design of multicentre studies (Cutler, 1982, Abel, 1988, Soloway, 1989).

Within this prospect, in Chapter V we performed a prognostic analysis of a controversial subgroup of superficial bladder carcinomas. Chapter VI describes a prognostic analysis of superficial bladder carcinomas treated with intravesical instillations, indicating the importance of side-studies on prognostic factors in every clinical trial.

5. Prognostic factors in renal cell carcinoma

5.1 Introduction

Renal cell carcinoma (RCC) is characterized by its unpredictable behaviour and tendency to recur and metastasize even several years after diagnosis. It is a neoplasm with, in general, an unfavourable prognosis. Thirty percent of the patients already have metastases at the time of diagnosis and only 50% of the patients present with a localized disease and have a chance to be cured (de Kernion, 1978). Depending on the stage of the tumor, the 5-years survival rate ranges from 30 to 60% (Holland, 1973). In metastatic disease the prognosis is very poor with a one year survival of 26% (Patel, 1977).

Surgery remains the corner stone in the treatment and represents the starting point. It remains the only possibility for cure in patients in whom the tumor is confined to the kidney. Patients with advanced disease at the time of diagnosis suffer from the consequences of incurability. Therefore, patients with disseminated cancer need adjuvant treatment to improve survival.

The efficacy of adjuvant treatments like chemotherapy and immunotherapy still has to be elucidated further. Especially immunotherapy has shown promising results (Horoszewics, 1989, Muss, 1991). These therapeutic options with low response rates have the disadvantage of considerable side effects in patients who are often already physically disabled by the metastatic disease. Strict indication for adjuvant treatment is therefore of importance in order to avoid useless and harmful treatment.

With the use of prognostic factor analysis we are able to individualize the treatment of cancer patients. From the onset of treatment, it became clear that not all had the same clinical course. Several staging systems have been developed and tested for practical use in order to predict the clinical course of each patient. The staging system initiated by Flocks and Kadesky and popularized by Robson and associates, appeared to be a reliable indicator for survival (Flocks, 1958, Robson, 1969). Another staging system which includes the descriptive tumor, nodes, and metastases (TMN) characteristics has been developed by the International Union against Cancer and appeared in its last edition in 1987 (Beahrs, 1988). A comparison of the two classification systems with the TMN stages is shown in Table 8.

Table 8 Comparison of the two classification systems with the TMN stages

	TNM	IUCC	Robson
Small tumor, no enlargement of kidney	T1	1	A
Large tumor, cortex not broken	T2	2	A
Perinephric or hilar extension	T3	3	B
Extension to neighbouring organs	T4	3	D
Nodal invasion	N+	3	C
Renal vein involved	V1	3	C
Vena cava involved	V2	3	C
Distant metastases	M+	4	D

The applicability of these systems has been subject to several investigations, and in different subsets of patients they appeared to be good predictors for survival (Bassil, 1985, Hermanek, 1990) Especially the IUCC staging system is a reliable indicator of the extent of the tumor (Hermanek, 1990). Classification systems are therefore at the basis of treatment in clinical practice. As adjuvant therapy becomes increasingly popular, the ability to identify patients at risk for progression is critical. Another advantage of the use of a standardized classification model is the ability to exchange treatment results. The classification models are also used for a more precise prognostic factor analysis. It is important to determine whether these new factors are of additional prognostic significance to the prognostic characteristics already known.

5.2 Clinical and biochemical prognostic parameters

Gender has been indicated as a prognostic parameter. McNichols et al. observed a better survival in women with RCC (McNichols, 1981). The same was seen in an investigation in young (20 to 40 years) patients (Lieber, 1981). This is in contradiction with Selli et al., who could not find any relation (Selli, 1983).

Age is also not a clear prognostic parameter. Griffith said that "the extremes of age are usually regarded as of bad prognostic import, youth because of increased activity in the growth, and old age from the shorter natural extension of life" (Griffith, 1964). This is confirmed in some investigations where a worse prognosis was seen in elderly patients (Selli, 1983). However, Neves et al. did not found any relationship between age and survival (Neves, 1988).

The symptoms of patients with RCC varies. Performance status has been described for several decades as a measure for the evaluation of the effects of chemotherapeutic drugs (Karnofsky, 1949). Also, in patients with metastatic RCC the performance status proved to be a powerful independent prognostic factor (Maldazys, 1986). Another symptom which showed to be of prognostic significance is a history of weight-loss. In several studies this event, which occurs before the diagnosis is established, showed to be a bad prognostic sign (Lieber, 1981, Selli, 1983, Neves, 1988). Fever at the time of diagnosis is also, though rarely mentioned, a bad prognostic feature (Lieber, 1981).

From the laboratory results some prognostic parameters were obtained. Especially erythrocyte sedimentation rate (ESR) has been investigated for a long period. Several investigators described a worse prognosis in patients with an elevated ESR at the time of diagnosis (Bech Hansen, 1972, Hannisbal, 1989). However, this could not be confirmed by Lieber et al. in their selected young adult population (Lieber, 1981). In an investigation done by Chasan et al. the level of serum calcium was of no influence on survival (Chasan, 1989).

5.3 Anatomical prognostic parameters

Nephrectomy will be performed in almost every patient suspected to have a RCC. In case of a localized tumor, nephrectomy is mandatory in order to obtain cure for the patient. In more advanced cancers a debulking operation may be beneficial for local control of the neoplasm or as a basis for protocols on adjuvant treatment (Golimbu, 1986A, Horoszewics, 1989, Giuliani, 1990).

As has been mentioned in the introduction of this chapter the basis of dividing patients in prognostic groups is the TMN classification system. Numerous reports obtained positive prognostic significance for tumor stage, or the presence of metastases in bone, soft tissues and lymphnodes (Fuselier, 1983, Nurmi, 1984, Bassil, 1985, Golimbu, 1986A).

In a report from Priestly, published in 1939, tumorweight showed to be of prognostic significance (Priestly, 1939). Nowadays the relation between tumor size and clinical behaviour of the carcinoma is clear. This finding was already mentioned in 1970 (Bottiger, 1970). Size of the primary tumor is also obviously a predictor for the risk of

metastasis (Fuhrman, 1982).

In every study concerning prognosis the M-stage has been mentioned as the strongest factor (Siminovitch, 1983). Also the site of the metastases appeared to be of prognostic significance. Patients with lung metastases only, react better on chemo- or immunotherapy than patients with metastasis to other single organs (e.g. bone) or multiple organs (de Kernion, 1983). Therefore the survival of patients with only lung metastases is better (Maldazys, 1986).

Another feature, which has been clarified, is the bad prognostic sign of regional lymphnode metastases (Middleton, 1973). Several investigation found a positive effect of a radical lymphadenectomy on prognosis (Giuliani, 1990). This effect on prognosis, however, is still controversial.

One of the supplements of the TNM classification model, which is included in the IUCC staging model of 1987, is the renal vein involvement. The relation between renal vein involvement and risk on distant metastases and therefore the influence on survival has already been mentioned in 1953 (Throckmorton, 1953). The extent of this involvement adversely affects prognosis in several investigations (Skinner, 1971, Siminovitch, 1983). Also invasion of the vena caval wall seems to be a bad prognostic sign. However some other investigations found no additional prognostic information of renal vein involvement on survival (McNichols, 1981, Fuselier, 1983, Nurmi, 1984, Ferrari, 1990). It is now clear that the survival rate of patients with vena cava involvement is not worse if it can be removed radically (Golimbu, 1986B, Hatcher, 1991).

5.4 Pathological, cytogenic and karyometric prognostic parameters

The histology of renal cell tumors must imply the definition of carcinoma. Grawitz gave the first definition of this carcinoma (RCC), using embryological and architectural characteristics (Grawitz, 1884). Later, the differentiation into other cell-types, like adenomas, sarcomas and oncocytomas, have been studied (Foot, 1951). Of course these differentiations will have implications on prognosis. It is therefore that we restrict the "renal tumor" only to the true carcinomas (RCC). Within this group certain histological characteristics are of prognostic importance. The prognostic significance of histological grade has been known for over fifty years (Priestly, 1939). In the beginning of this century grading according to Hand and Broders was done, and this appeared to have implications

on survival (Hand, 1932). Most grading systems are nowadays according to the WHO (Mostofi, 1981). This describes nuclear atypia, including the size of nucleoli, supplemented by some cytoplasmic features. It appears, however, that this grading system suffers to some degree from interobserver variability. It is probably for this reason that modifications have been developed in an attempt to simplify the criteria of grade (Foot, 1951, Skinner, 1971). The use of various grading systems, accepted so far in the practice of pathology, will not be beneficial for comparison of results. Another problem in grading is the heterogeneity of the tumor. When several sections of one tumor are investigated, there is often a great variability in morphology from section to section. It is also for this reason that the importance of grade for prognosis is diminishing. This problem with histological characterization is a challenge for many investigators to simplify the grading system. Reis et al. looked for a more reproducible way of grading by using the histologic pattern as a prognostic factor (Reis, 1988). The implications on prognosis of these architectural patterns are however controversial, especially for papillary neoplasms and carcinomas which are predominantly composed of granular cells (Fryfolge, 1948, Murphy, 1965, Mancilla-Jimenez, 1976, Fuhrman, 1982). However there are some characteristics which are related to survival: patients with spindle carcinomas have a poor prognosis (Tomera, 1983). Medeiros et al. showed a clear relation between various cell types and grade, and described an influence on survival (Medeiros, 1988). In a recent report Delahunt et al. showed a new histologic marker, the nucleolar organizer region, which is visible by electron microscopic examination and appeared to be of prognostic significance (Delahunt, 1991). It can be expected that in the near future more of these features will be described and may become of use in daily clinical practice.

A new tendency in histologic characterization is the nuclear morphometry. Morphometric analysis has been used successfully to asses the outcome of patients with malignancies when standard pathologic grading systems failed. Several parameters were investigated, including nuclear elongation, nuclear roundness, nuclear crowding, mitotic density and tumor grade, and evaluated for their accuracy in predicting prognosis. Several reports showed a positive correlation between these various morphometric parameters and survival in a subdivision of patients with stage I disease (Tosi, 1986, Bibbo, 1987, Murphy, 1990). Gilchrist et al. described nuclear size as a prognostic factor in all RCC (Gilhirst, 1984). They concluded that larger nuclei were related with a shorter survival.

Flowcytometry is known as an easy and reproducible method to determine deoxyribonucleic acid (DNA) content. This technique can be used in every clinical practice. Otto et al. concluded in their study a positive indication of the DNA content on risk for recurrence and advised adjuvant therapy after nephrectomy in bad risk selected patients (Otto, 1984). A higher prognostic significance could be obtained when combining flowcytometry with nuclear grading. DNA content in RCC might be a superior prognostic indicator than other clinical and microscopical parameters (Ljungberg, 1986). These results were confirmed by Rainwater et al. in a study with a long follow-up (Rainwater, 1987). On the other hand, another investigator could not find a prognostic significance of flow cytometry as a significant TMN stage-independent impact on prognosis and re-opened the discussion about the widespread clinical application of ploidy status (Currin, 1990).

With all the prognostic factors known today it still not possible to predict the outcome of patients with a RCC correctly. To get a better insight in the value of each prognostic factor a review of all clinical and histological parameters is mandatory, and especially an evaluation of each value is of interest.

Table 9 shows an overview of the mostly used prognostic parameters in RCC.

Table 9: + = significant correlation, - = no significant correlation

	McNichols '81 n=506	Fuselier '83 n=161	Selli '83 n=115	Siminovit '83 n=246	Nurmi '84 n=257	Bassil '85 n=252	Storkel '89 n=431	Giuliani '90 n=200
sex		-	-		-			
age			+		-		+	
weight loss			+					
T	+	+	+	+	+	+	+	+
N	+	+		+	+	+		+/-
M	+	+	+	+	+	+		+
V	-	-	-	+	+	+		+
tumor size					-			+
grade	+	+	+		+		+	+
cell type	-	-	+		-		+	-

In chapter VII an overview of all possible prognostic factors, which can be used

in daily clinical practice for patients with RCC, is given. By using the results of chapter VII a study of a low-risk group of patients with RCC is performed in order to detect the patients at risk. The results are shown in chapter VIII.

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CHAPTER II

THE IMPORTANCE OF PROGNOSTIC FACTORS IN THE INDIVIDUAL TREATMENT OF PATIENTS WITH DISSEMINATED GERM CELL TUMOUR.

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Following chemotherapy for disseminated testicular cancer, 55 patients underwent surgery because of residual tumour. The histological findings were viable tumour in 12 patients, mature teratoma in 12 and fibrosis and/or necrosis in 31. Retroperitoneal abdominal masses were evaluated radiographically before and after chemotherapy. The reduction in size of these masses after chemotherapy appeared to have prognostic significance. A decrease of more than 70% was always associated with fibrosis. A residual mass over 50 mm indicated viable tumour or mature teratoma. Seminoma or embryonal carcinoma was more likely to result in fibrosis/necrosis in the resected tissue. Both the Indiana and the EORTC classification models can be used for prognosis. Radiographic measurements before and after chemotherapy are of considerable prognostic significance. These objective indicators help in planning treatment and so diminish side effects of therapy and maintain or even increase the high cure rate in disseminated testicular cancer.

INTRODUCTION

Testicular cancer should now be curable even in an advanced stage. Cure rates of 70-80% have been reported for disseminated germ cell tumors of the testis. The introduction of cisplatin-based combination chemotherapy has increased cure rates dramatically (1,2). Improvements in staging methods and surgical techniques are also relevant to cure rates (3,4). Despite the encouraging results, we need a better insight into the timing and extent of the different phases of treatment.

The European Organization for Research and Treatment of Cancer (EORTC) classification of prognostic factors and the Indiana classification of extent of disease, define risk factors for individual patients at the start of treatment (5,6). The number of cycles of chemotherapy is, however, usually prearranged and sugsequent surgery is standard when residual tumour is present.

The purpose of this study was to look for other prognostic factors that might help to define a more individual treatment schedule and thus to diminish the side effects of therapy and increase cure rates.

PATIENTS AND METHODS

Between January 1979 and January 1988, primary chemotherapy was given to 61 patients with disseminated germ cell tumours. The histology of the primary tumour was non-seminoma in 46 cases and seminoma in 15. 3 of these were extragonadal tumours. The extent of disease was established according to the Royal Marsden classification (7). Included were stages IIc, III, and IV. Chemotherapy before surgery consisted of 4 or more cycles of cisplatin-based combination therapy (cisplatin, vinblastine, bleomycin (PVB), bleomycin, etoposide, cisplatin (BEP) or PVB and BEP alternating); 51 patients received 4 cycles of chemotherapy; 10 patients received up to 8 cycles before surgery because tumour markers remained high. The patients were staged retrospectively according to the Indiana classification of extent of disease and the EORTC classification of prognostic factors (5,6).

After chemotherapy, all patients were evaluated by clinical, biochemical, and radiological examination. Biochemical assessment included serum α-fetoprotein (α-FP,

normal <20 ng/ml) and human chorionic gonadotropin (ß-HCG, normal <3.0 ng/ml). Radiological examination consisted of abdominal computed tomography (Siemens Samatron DR3), chest X-ray and lung tomography. Radiological re-evaluation was done by one radiologist (C.B.). The cure rate, 3-year survival rate and histopathological outcome of surgery were established in relation to the 2 classifications. Following chemotherapy, surgery was performed in the 55 patients with residual tumour. Residual mass was defined as nodes >10 mm and/or residual strands on computed tomography. Five patients had no residual mass after chemotherapy and normal tumour markers; they were followed up closely. One patient died soon after establishment of the diagnosis. The operation in patients with residual mass after chemotherapy consisted of retroperitoneal lymph node dissection (RPLD) in 44 patients, thoracotomy in 6 and both in 5 patients. Resected residual tumours were examined histologically. Patients with fibrosis or mature teratoma were followed up closely at lengthening intervals. Patients with viable tumor in the resected tissue were given additional chemotherapy.

The size of metastatic tumour before and after chemotherapy was measured radiologically (largest transverse diameter) and compared with histopathological assessment.

Cure was defined as absence of biochemical or radiological evidence of disease after chemotherapy or as a surgical finding of fibrosis or mature teratoma (the latter only if all residual tumour mass had been removed).

Follow-up ranged from 5 months to 8 years (mean 3 years).

The accuracy of the classifications was analysed statistically by the Kaplan-Meier method for survival and cure rates, and significance was estimated with trend tests. Differences in histologic outcome were analysed with the chi-square test; in the case of continuous variables, the Kruskal-Wallis test was used.

RESULTS

The overall 3-year survival rate of 61 patients with disseminated germ-cell cancer was 78% and the cure rate 72%. Patients were placed in the subgroups of the EORTC classification (good, moderate or bad prognosis) and of the Indiana model (minimal, moderate, advanced disease). Survival and cure rates were calculated for the patients in

the subgroups of the 2 classifications (Tables 1 and 2). Both methods of classification correlated well with cure and survival rates.

		No.	cure rate (%)	
- EORTC	good	18	84	
	moderate	30	77	P=0.029
	poor	13	62	
- Indiana	minimal	17	88	
	moderate	28	79	P=0.017
	advanced	16	69	

Table 1 Prognostic Classification and Cure Rate

		No.	3-years survival (%)	
- EORTC	good	18	100	
	moderate	30	78	P=0.006
	poor	13	51	
- Indiana	minimal	17	89	
	moderate	28	76	P=0.09
	advanced	16	69	

Table 2 Prognostic Classification and Survival

The histologic assessment of specimen was of great importance for 3-year survival: 31 patients had fibrosis or necrosis or both, 12 had mature teratoma, and 12 had viable tumour. The 3-year survival rates for these groups are shown in Table 3. The histology of the primary tumour was important for the results of surgery; seminoma or embryonal cell carcinoma was more likely to produce fibrosis or necrosis (Table 4). Two patients with fibrosis died: 1 from brain metastases and 1 from a malignant Leydig -cell tumour. Only 1 patient died post-operatively after resection of a 30-cm abdominal tumour (mature teratoma). Of the 12 patients with viable tumour in the residual mass, 7 died from testicular cancer. The 5 patients with no residual tumour after chemotherapy and

who were not operated on are still alive with no evidence of disease.

	No	3-years survival rate (%)	
Fibrosis	31	93	
Teratoma	12	92	P<0 001
Tumour	12	27	

Table 3 Histological Results and Survival

	No.
Seminoma	9
Malignant teratoma undifferentiated	4
Malignant teratoma	1
Malignant teratoma trophoblastic	1

Table 4 Histology of Primary Tumour in Fibrosis Group

The relative reduction in tumour mass after chemotherapy had great significance. The median decrease was 70% in patients with a histological outcome of fibrosis and 28% in the group with mature teratoma or tumour (Table 5). The difference was statistically significant.

	median size (mm) before Chemotherapy	Surgery	Median difference Absolute (mm)	Relative (%)
Fibrosis	75	20	40	70
Teratoma	63	53	3	10
Tumour	100	53	40	50
	P=0 08	P<0 001	P=0 003	P<0 001

Table 5 Size of Tumour and Results of Operation

In 5 patients the abdominal mass increased during chemotherapy; 4 had mature teratoma and 1 had viable tumour. We found that a reduction of more than 70% was always associated with a histological report of fibrosis (Table 6).

Decrease	No.	Fibrosis	Teratoma	Tumour
>30%	36	28	2	6
>50%	24	19	2	3
>70%	15	15	0	0

Table 6 Relative Decrease on CT Scan and Histological Assessment

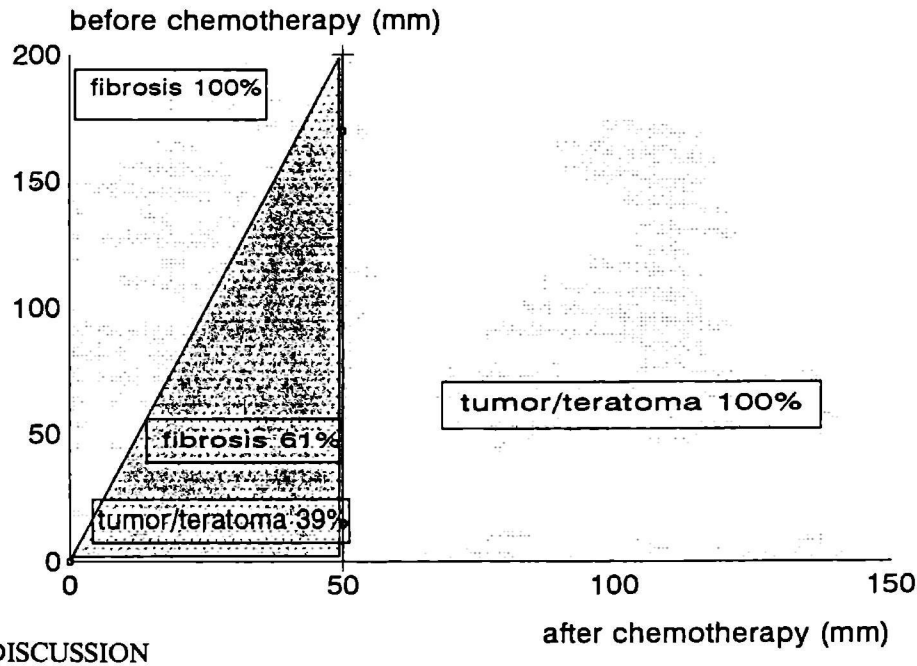
The size of residual mass after chemotherapy and the histological assessment are shown in Table 7. Even in small residual tumours, viable tumours could be found, although rarely. Large residual tumors were more likely to contain mature teratoma or viable tumour. This was always the case for residual masses >50 mm.

Size (mm)	No.	Fibrosis (%)	Teratoma (%)	Tumour (%)
0-20	20	85	10	5
21-30	9	78	11	11
31-50	9	56	22	22
>50	9	0	55	45
Total	47	62	22	16

Table 7 Size and Histological Assessment

When Table 5 and 6 are combined, the results shown in the Figure are obtained. Elevated tumour markers (α-FP and/or ß-HCG) following chemotherapy were also of prognostic importance. Three patients with persistently high α-FP or ß-HCG after chemotherapy underwent surgery. They all had viable tumour in the resected tissues, but 9 patients with normal levels tumour markers still had viable tumour in the resected tissues.

Fig 1: size of the abdominal mass before and after chemotherapy



DISCUSSION

Disseminated testicular cancer can be cured in most cases with a combination of chemotherapy and surgery. It is, however, difficult to predict the results of treatment in individual cases. The EORTC classification of prognostic factors and the Indiana classification of extension of disease attempt to categorise patients in order to predict the efficacy of treatment. Multivariate analysis leads to the formation of prognostic groups that indicate response rates and survival rates (5,6,8,9). In the present series the EORTC classification was more appropriate. Standard therapy for disseminated testicular carcinoma is the same, whatever the risk: 4 cycles of combination chemotherapy eventually followed by resection of residual mass. The detection of prognostic factors should result in more individualised treatment.

Individualised chemotherapy might consist of fewer cycles for patients with good prognostic factors and more cycles (with more effective and probably more toxic chemotherapy) for those at high risk.

With regard to the best individual treatment, the role and timing of cytoreductive

surgery are of special interest. Standard treatment includes surgery when a residual mass remains after chemotherapy (10,11), but should every patient with a residual tumour, however small, undergo surgery?

Survival rates depend largely on the histology of resected specimens (12,13). A patient with residual viable tumour or mature teratoma should undergo surgery in order to reduce the size of the tumour and make it accessible to secondary chemotherapy, since it could grow or become malignant again (14,15). Fibrosis or necrosis need not to be resected.

Donohue et al showed that histology of the primary tumour was an important prognostic sign for the RPLD (16). Tumours without teratomatous elements were more likely to lead to fibrosis in the resected tissue. Our data also show that the absense of teratomatous elements in the primary tumour are more likely to produce fibrosis in the residual mass, especially when combined with a marked reduction in size of the abdominal mass during chemotherapy.

Unlike other workers we found that the residual mass after chemotherapy for disseminated pure seminoma could contain viable tumour (17,18). This was found in 4 of 15 patients with pure seminoma. Motzer et al reported similar results (19). The reduction of retroabdominal metastatic tumour after chemotherapy appeared to be of value. A relative decrease of more than 70% was always associated with fibrosis in our patients. The reduction was significantly less in the group of patients with residual viable tumour or mature teratoma. A residual mass >50 mm was associated with mature teratoma or viable tumour. Mature teratoma was often found after an increase in metastatic mass during chemotherapy. However, even in the smallest residual mass, viable tumour cells could be present. Other investigators could not find such a clear correlations between radiological findings and histological outcome (20,21).
Although we operated on a relatively large number of patients after chemotherapy, it is unwise to generalise from a small study group. Prospective studies with large numbers of patients will give more insight into this important issue.

It was concluded that the relative reduction in size of metastases in patients on chemotherapy and the size of the residual mass after chemotherapy are significantly related to histological outcome; the histology of the primary tumor is also of prognostic importance. Prospective trials should be undertaken to find out if chemotherapy could

be reduced when there is a rapid reduction in tumour volume and no teratomatous elements in the primary tumour, and if prolonged chemotherapy might obviate the need for surgery in other cases by bringing about complete remission.

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CHAPTER III

ANALYSIS OF PROGNOSTIC FACTORS IN DISSEMINATED PROSTATIC CANCER
AN UPDATE

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A statistical analysis of prognostic factors in 175 patients with hormonally treated disseminated prostatic cancer was done. The prognostic significance of performance status (PS), hemoglobin (Hb), alkaline phosphatase (AlkP), and testosterone was assessed with a univariate analysis. The authors did not find significant prognostic value in age, tumor size or grade, prostatic acid phosphatase, or prostate-specific antigen in these patients. In a multivariate logistic model (Cox regression), PS, Hb, and AlkP were found useful for dividing patients into prognostic groups. The prognosis for high risk patients on standard hormonal treatment was very poor.

The authors concluded that research on prognostic factors is useful and permits a division of patients into risk groups that makes choice of treatment more accurate. The use of new treatment combinations at the start is appropriate for high risk patients with disseminated prostatic cancer.

INTRODUCTION

Since the development of "androgen deprivation" by Huggins, et al., over 45 years ago, hormonal manipulation has been the best available treatment for patients with disseminated prostatic carcinoma (1). The improvements brought about by new methods of hormonal deprivation are mainly a better subjective response and reduction of side effects. But sofar no important prolongation of survival has been observed. Several methods of mechanical or chemical castration have been used, especially recently. However the value of chemotherapy is limited, partly by its poor effectiveness and its side effects (2,3). New kinds of therapy are therefore needed, especially for patients who are refractory to the present ones.

Extended knowledge regarding prognostic factors is essential for the design of future studies. It is important to be able to divide patients into risk groups, so that we can select appropriate therapeutic options. On basis of a retrospective study we analyze here the value of various prognostic factors in the selection of treatment for patients with disseminated prostatic cancer.

PATIENTS AND METHODS

Between September 1984 and January 1988, 191 patients entered two trials organized by the Dutch South Eastern Urologic Oncology group: 75 patients received luteinizing hormone-releasing hormone (LHRH) as a monthly depot (Zoladex, ICI, London), and 116 patients underwent orchiectomy with or without administration of an anti-androgen (Anandron, Roussel, Paris) (4,5). The results of the two trials were similar in hormonal deprivation, and the groups were pooled for analysis of prognostic factors. Of the 191 subjects, 16 with advanced, but local, disease were excluded; 175 patients with bone metastasis (M+, according to the TMN classification model) remained.

Pretreatment factors analyzed for prognosis were age, performance status (PS, Karnofsky score), tumor size (T according to the TMN classification, with rectal palpation and transrectal ultrasonography), and grade (according to Mostofi, 6). Laboratory results included were hemoglobin (Hb; in anaemia Hb < 8.5 mmol/L),

alkaline phosphatase (AlkP), prostatic acid phosphatase (PAP), prostate-specific antigen (PSA), and testosterone. PSA was determined with the immune-enzymetric Tandem-e-psa assay (Hybritech). Testosterone was measured in 75 patients who were in a pharmacokinetic clinical trial; 59 with M+ were evaluated. Because the patients were treated at different centers, laboratory AlkP and PAP results were standardized and classified relative to the upper limit of the normal range.

A patient was declared to have progression if his area of bone lesion involvement (entire body) increased more than 25% or the number of bone lesions increased, as compared to the best response.

Statistical methods:

Time to progression and survival, calculated from the start of treatment, were used as end points in this study. Survival was considered with respect only to death related to cancer. To assess the influence of the mentioned above factors, the Kaplan-Meier method was used for estimation, and the log-rank test was applied for statistical testing. In a multivariate analysis using the proportional-hazard model (Cox regression), we analyzed the variables simultaneously with a stepwise procedure (on level $p = 0.10$) (7).

RESULTS

During the period of follow up (mean, 2.3 years), 92 of 175 patients had progression on hormonal treatment. Of those 92, 82 died, 68 because of prostatic cancer.

The results of univariate analysis of prognostic factors are shown in Table 1.

Factor		No.	2-yr survival (Kaplan-Meier)	P value (log-rank test)
Age	<60yr	10	0.34	0.44
	60-70yr	58	0.64	
	70-80yr	80	0.61	
	>80yr	27	0.48	
T	0	19	0.45	0.70
	1	18	0.47	
	2	31	0.47	
	3	46	0.58	
	4	59	0.65	
Grade	1	21	0.69	0.17
	2	74	0.63	
	3	74	0.62	
PS	100%	43	0.72	0.006
	80-90%	73	0.65	
	60-70%	41	0.43	
	0-50%	14	0.51	
Hb	>8.5 mmol/l	83	0.76	<0.001
	<8.5 mmol/l	90	0.48	
AlkP	normal	58	0.76	<0.001
	-2.5 x U/l	57	0.51	
	more	57	0.47	
PAP	normal	22	0.74	0.16
	-2.5 x U/l	36	0.61	
	-10.0x U/l	45	0.62	
	more	71	0.55	
PSA	<100 micrg/l	30	0.79	0.74
	100-300	31	0.62	
	300-800	37	0.48	
	>800 micrg/l	34	0.47	
Test	<300 ng/dl	17	0.49	0.024
	>300 ng/dl	42	0.74	

TABLE 1 Univariate Analysis of Prognostic Factors
PS: performance status (Karnofsky score); Hb: hemoglobin; Alk P: alkaline phosphatase; PAP: prostatic acid phosphatase; PSA: prostate-specific antigen.

The values of prognostic factors were the same for time to progression during hormonal treatment and for the survival, so only the values for survival are given. Bad

PS, anemia, and high AlkP were significantly related to shorter survival. Low testosterone concentration at the start of therapy was also associated wit poor prognosis. Histologic grade was not significantly related to survival. PAP only showed a numerical trend. As for PSA, no significant relation to prognosis could be detected in these patients with disseminated prostatic carcinoma, although there was a trend to better 2-year survival with lower initial value of the antigen. We could not find a relation of age and T category at the beginning of therapy to survival.

After this univariate analysis, we did a multivariate analysis according to the Cox-regression model. All the variables (Table 1) were included in a stepwise analysis. The results are given in Table 2.

Factor		ß	P value
Hb	<8.5 mmol/L	0.50	0.07
AlkP	>1.25x upper limit	1.14	<0.001
PS	<100%	0.85	0.018
	< 60%	0.95	0.022

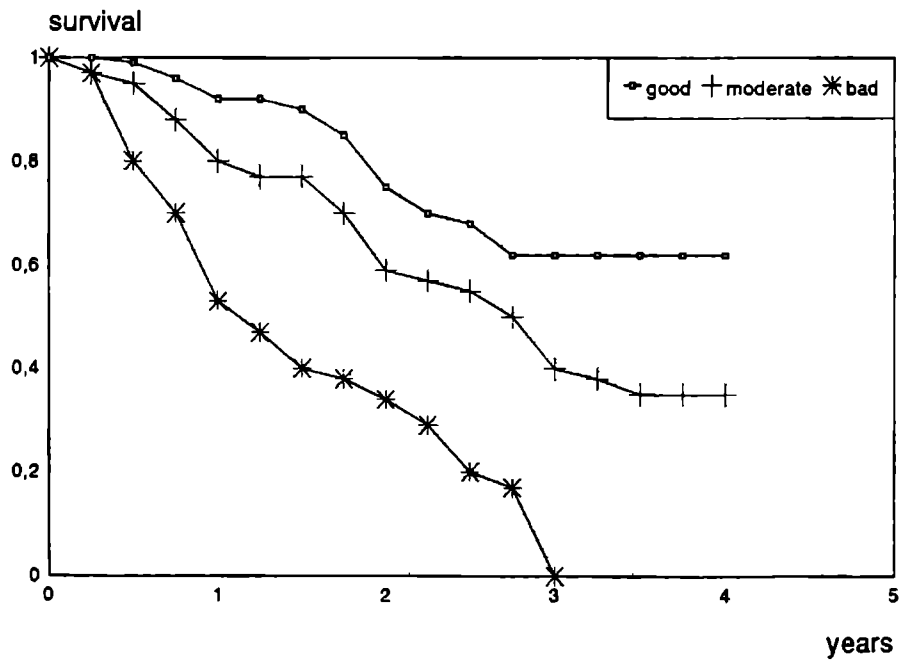
TABLE 2. Results of Cox Regression Model
Hb: hemoglobin; Alk P: alkaline phosphatase; PS: performance status (Karnofsky score)

PS, Hb, and AlkP were useful for obtaining prognostic groups. The other variables did not give additional information for these groups, although for testosterone this could be due to the relatively small number of patients (59) in whom it was measured. We were able to form three groups (good, moderate, and bad) for predicting the duration of survival.(Table 3 and Figure 1).

Good (n = 64)	AlkP < 1.25 x upper normal value and PS = 100% AlkP < 1.25 x upper normal value and PS = 60-90% and no anemia
Moderate (n = 46)	Not in good or bad group
Bad (n = 57)	AlkP > 1.25 x upper normal value and PS < 50% AlkP > 1.25 x upper normal value and PS 60-90% and anemia

TABLE 3. Prognostic Groups
Alk P: alkaline phosphatase; PS: performance status (Karnofsky score).

Figure 1: prognostic groups



At least 20-30% of patients with disseminated prostatic cancer do not react to hormonal treatment, and an equal percentage becomes resistant to the treatment within 2 years (8). New treatments are needed for these subgroups of patients. Possibilities of new hormonal and cytotoxic treatment were reviewed earlier in this journal (9,10,11). To identify patients with a poor prognosis before the start of treatment, analysis of factors that influence survival are useful.

In our study, PS, Hb, AlkP, and testosterone were found to be of prognostic significance. The importance of PS and Hb has been reported by Berry et al., but they used these factors for hormonally unresponsive prostatic cancer treated with chemotherapy (12). The unfavorable influence of high AlkP on survival was remarkable and has been established by others (13,14,15). According to Wilson et al., a low testosterone concentration at the start of hormonal treatment is an adverse prognostic sign (16). PAP is generally accepted as having prognostic significance, but we could not find a significant influence of PAP on survival in our patients (17,18). PSA is widely accepted as the most important factor for detection of prostatic cancer and monitoring of its treatment (19,20). In our patients with disseminated carcinoma, PSA did not influence survival significantly, although there was a steady decrease in survival with increasing PSA. Comparable results were obtained by Kuriyama, et al. (21). Age was not a significant prognostic factor in our study. That result confirmed the result of Harrison, but Wilson et al. detected a worse prognosis for patients under 60 and over 80 (22,23).

On the basis of our multivariate analysis, we defined three risk groups that react differently on hormonal treatment. High-risk patients included those with a disseminated prostatic cancer that is so far advanced that it worsens PS, results in anemia, and raises AlkP. Those patients should be subject to new kinds of treatment; the use of currently accepted standards should be questioned. That is in view of the expected early escape from hormonal therapy, which is more likely to occur with their more aggressive tumors. It is logical to select this group of patients to examine whether early combination of hormone and cytotoxic therapy or radiotherapy is superior to hormonal manipulation alone. The aim of the combination is to prevent

growth of both hormone-dependent and hormone-independent tumor cells from the beginning and to lengthen time to progression and therefore improve survival (24).

In summary, we conclude that treating patients with disseminated prostatic cancer in a general way does not justify the heterogeneity of response to standard therapy. By statistical analysis, we established the importance of prognostic factors for forming risk groups. Especially for the high risk group, innovative treatment is needed.

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CHAPTER IV

THE VALUE OF BIOCHEMICAL MARKERS IN THE MANAGEMENT OF DISSEMINATED PROSTATIC CANCER

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The biochemical markers alkaline phosphatase (Alk P), prostate acid phosphatase (PAP), and prostate-specific antigen (PSA) were measured 3-monthly in 61 patients with disseminated prostatic cancer who were treated with LHRH analogues. The decrease in Alk P and PSA during the first 6 months of treatment was significantly related to a better survival. In this follow-up study only PSA was useful for monitoring of prostatic cancer during hormonal treatment. Before it was visible on a bone scan, PSA gave an indication of tumor progression. PSA might permit omission of routine bone scanning. Consensus must be obtained about the cost-saving use of biochemical markers in the treatment of disseminated prostatic cancer. With the number of treatment options increasing, objective measures are of utmost importance. Biochemical markers can be used for prognosis and monitoring of the treatment of patients with disseminated prostatic cancer.

INTRODUCTION

Prostate cancer is one of the major causes of death in older men in western countries (1). Hormonal therapy has been used for disseminated prostatic cancer for some 50 years and is still the treatment of choice (2). During the last few years, an increasing number of new treatment regiments have been studied, in an attempt to improve objective and subjective responses and a reduction in side effects. Several possible hormonal and cytotoxic treatments have been evaluated, but no spectacular improvements in survival have appeared (3-5).

The design of future studies will require extensive knowledge of prognostic factors that will give objective information on the response to treatment and will be helpful in monitoring patients with prostatic cancer. For practical and economic reasons, it is useful to derive those prognostic factors from laboratory results. Within this context, we studied the known factors prostate-specific antigen (PSA), alkaline phosphatase (Alk P), and prostate acid phosphatase (PAP), with regard to the prognosis of patients with metastatic prostate cancer and the monitoring of their tumors during hormonal treatment.

PATIENTS AND METHODS

Between September 1984 and January 1988, 61 patients with newly diagnosed metastatic (M+) prostate cancer received luteinizing hormone-releasing hormone (LHRH) as a monthly depot (Zoladex, ICI) (6). These patients were part of a pharmacokinetic study which was organized by the Dutch South Eastern Urologic Oncology group. Pretreatment values of PSA, Alk P, and PAP were measured. During the follow-up these values were measured every 3 months. Liver function tests were performed to ensure that the Alk P increase was due to bone metastases. Because the patients were treated at different centres, Alk P and PAP were standardized and classified relative to the upper limit of the normal range. PSA was determined with the immune-enzymetric Tandem-e-psa assay (Hybritec, San Diego, Calif., USA) in one laboratory.

A patient was considered to have progression if the area of bone lesion involvement (entire body) increased by more than 25% or if the number of bone lesions

on bone scintigraphy increased as compared with the best response.

Statistical Methods

We used time to progression and survival, both calculated from the start of treatment, as end points in this study. Survival was considered with respect only to cancer-related death. To assess the utility of the 3 biochemical markersm, we used the Kaplan-Meier method for estimation and the log-rank test for statistical testing. Where appropriate, a chi-square test was applied.

RESULTS

During the follow-up (mean 3 years), 36 of the 61 patients had a progression. Of those 36, 31 died, 26 deaths were due to the prostate cancer.

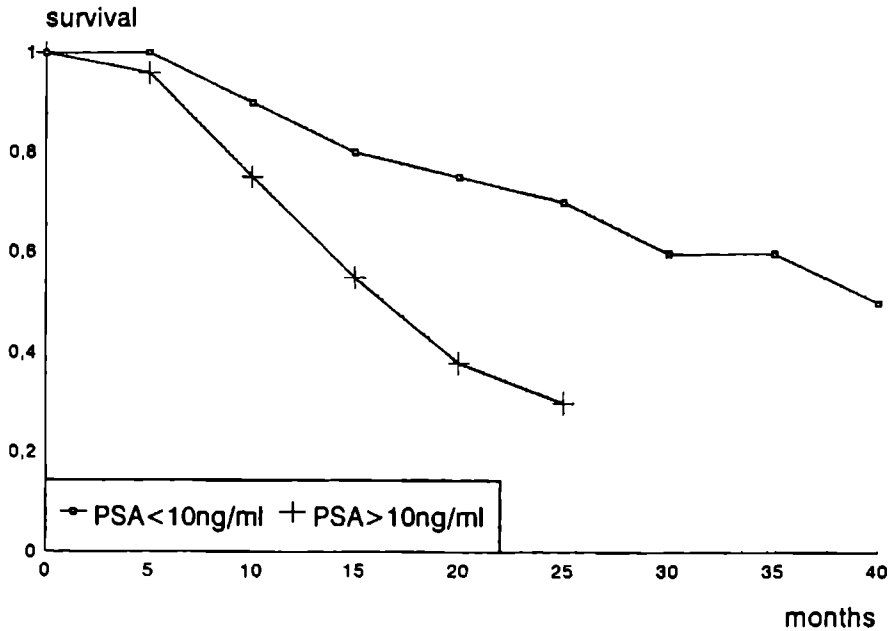
Six months after the start of hormonal therapy the values of PSA, Alk P, and PAP were measured and compared with the pretreatment values. The relation between these examinations and progression is shown in table 1.

	n	time to progression (mo)	
PSA			
< 90% decrease	15	10	
> 90% decrease	43	27	p=0 024
Alk P			
decrease	31	30	
same/increase	28	20	p=0 70
PAP			
decrease	47	22	
same/increase	9	18	p=0 27

Table 1 relative change after 6 mo of treatment.

Time to progression and survival during hormonal treatment are equally related to the results, so only the values for progression are given. Of the 3 factors only a change in PSA showed a significant influence on time to progression, but only if there was a dramatic change in PSA (more than a 90% decrease). The absolute values of PSA, Alk P, and PAP after 6 months of treatment are shown in table 2. PSA under 10 ng/ml appeared to be related to a better prognosis. The clear difference is shown in figure 1.

Figure 1: PSA after 6 months of treatment



A relation could also be detected for Alk P; a normal value after 6 months of therapy was related to a better prognosis. We could not find significant results for PAP on this subject (Table 2).

		n	time to progression (mo)	survival (mo)
PSA	< 10 ng/ml	33	22.7	33.2
	> 10 ng/ml	26	12.5	18.8
			p= 0.0002	p= 0.041
Alk P	normal	33	21.1	31.0
	abnormal	26	14.4	19.7
			p= 0.0067	p= 0.0018
PAP	normal	37	19.9	28.2
	abnormal	20	15.5	19.4
			p= 0.12	p= 0.19

Table 2: Values after 6 mo of treatment in relation with progression and survival

We determined the values at 6 months after treatment as a new baseline for the three factors. Changes on this baseline within 3 months before tumor progression was seen on the bone scan were compared with the corresponding changes in patients whose tumors did not progress. Results are shown in table 3. We can see that only for PSA an increase compared with the baseline was significantly related to progression on the following bonescan. For Alk P and PAP, only trends are visible.

		change in biochemical factor		
	n	decrease	equal	increase
- Alk P				
progression	31	4 (12.9%)	15 (48 4%)	12 (38 7%)
no progression	11	1 (9.1%)	8 (72 7%)	2 (18 2%)
				p= 0 37
- PAP				
progression	30	2 (6.7%)	14 (46 6%)	14 (46.7%)
no progression	15	2 (13.3%)	10 (66 7%)	3 (20 0%)
				p= 0 07
PSA				
progression	25	5 (20 0%)		20 (80 0%)
no progression	15	7 (46 7%)		8 (53 3%)
				p= 0 02

Table 3 Changes from baseline values before progression measured on bone scan or last obtained values.

DISCUSSION

At least about 25-30% of patients with disseminated prostatic cancer do not react to hormonal treatment, and an equal percentage become resistant to the treatment within 2 years (8). With the use of prognostic parameters we are able to define these high risk patients. In a multivariate analysis Soloway determined a group of patients with a bad prognosis by using the extent of disease on a bone scan, serum testosterone concentration and performance status (10). In a recently published study, we defined a high-risk group for tumor progression and a 3-year survival of 0% by using the values of Alk P, hemoglobin, and performance status (9). Especially for such a high-risk group innovative

treatment is needed. Randomized trials are mandatory in the near future to establish new treatment options. Treatment options as early combination of hormonal and cytotoxic therapy or radiotherapy may prolong survival for these patients. Within this prospect factors that are of value for prognosis and for monitoring patients with advanced prostatic cancer during various treatments are increasingly important. The follow-up of patients with hormonally treated metastatic prostatic cancer consists of clinical, radiologic, and laboratory measurements. Bone scan investigation is still the standard in the follow-up and determination of progression. As for laboratory results, the three most widely investigated and used biochemical factors are PSA, Alk P, and PAP; these are relatively inexpensive. In our previous study we examined the prognostic significance of the pretreatment values of these three factors and the survival. Only the pretreatment value of Alk P was significantly related to shorter survival. Although there was an obvious trend between higher PSA or PAP and shorter survival, it was not statistically significant (9). Concerning PSA Kuriyama et al. obtained the same results in their original study (12). The value of Alk P has been neglected for many years. However, its prognostic value in patients with a positive bone scintigram has been established by many investigators (21-24). In this follow-up study, we found that a significantly longer survival was related to normal Alk P after 6 months of treatment. Alk P was not useful in monitoring of the cancer.

PAP has been extensively studied in relation to survival and time to progression. The prognostic significance of PAP has been used for many years (17,18). Since the introduction of PSA, the value of PAP has lost importance as a prognostic factor, especially in metastatic prostatic tumors (13,19,20). In our study, PAP was not of prognostic significance with regard to change during treatment, or monitoring of patients with metastatic prostatic cancer.

Important improvement has been made in the detection of PSA by Wang et al (11). Extended research has been done on the value of PSA for prognosis. PSA appeared to be of more importance for the detection and monitoring of prostatic cancer than as a pretreatment prognostic factor (9,12-15). The change in the value of PSA during hormonal therapy was of prognostic significance for survival. To obtain a better prognosis, a spectacular decrease in PSA is needed. An absolute value of PSA less than 10 ng/ml after 6 months of treatment was related to a prolonged time to progression and

therefore to survival. Cooper et al recently described similar results; the value of PSA must be in a normal range (< 10 ng/ml), although this was significant only in a quantitative analysis with the use of bone scintigraphy (16). Like others, we established the importance of PSA in monitoring hormonal treatment (12-15). In a significant number of patients, we saw an increase in PSA before we could see progression on a bone scan. Therefore, we believe that it is possible to monitor hormonal treatment with PSA and therefore omit the bone scan for routine follow-up examinations.

We conclude that prognostic measures are of major clinical importance in the management of patients with metastatic prostatic cancer. Clinical, pathologic, radiographic, and biochemical indicators are available and can be used not only to classify patients in prognostic groups, but also to assist in the selection of a therapeutic approach adapted to the characteristics of individual patients. With the number of treatment options increasing the importance of the availability of several prognostic factors is clear. Especially for patients with a poor prognosis innovative therapeutic approaches are needed to prolong survival. Therefore an improvement of objective measurements of treatment response is mandatory.

Factors like PSA, Alk P, and PAP are now used in every clinical practice in patients with prostatic cancer. They are relatively inexpensive. Taking into account the recent reports on the use of these biochemical factors and the results of our own study, we aim at reaching a consensus on their value in estimating the prognosis of disseminated prostatic cancer and in monitoring the results of its treatment. Before treatment, Alk P can be used as a prognostic indicator of time to tumor progression and therefore survival. PSA can be used in monitoring results of treatment; routine bone scanning might become unnecessary. More side studies in association with prospective randomised therapeutic trials dealing with these issues need to be performed before the value of the described biochemical markers over other investigations such as bone scan will be fully substantiated. Both in Europe (EORTC GU group) and in the United States such studies are in progress now.

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CHAPTER V

PROGNOSIS AND TREATMENT OF pT_1G_3 BLADDER TUMORS

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SUMMARY

Background: Patients with T1G3 bladder cancer have a considerable risk for recurrence and\or progressive disease. Until now no consensus has been achieved on the optimal treatment.

Methods: Within the Dutch South Eastern Bladder Cancer Study Group 155 patients with a T1G3 bladder tumor were seen between 1983 and 1988. After review of histology 121 could be evaluated and recurrence-free interval was studied with regard to prognostic factors. Prognostic factors such as sex, age, bloodgroup, abnormalities on intravenous urography (IVU), pretreatment tumor configuration, number of tumors, number of locations involved in the bladder, voided urine cytology, results of the random biopsies, and mitotic index were evaluated, using a multivariate analysis with the Cox proportional hazard model.

Results: During the follow-up period 70 (58%) patients had recurrent bladder cancer, and of these 30 (43%) had progression into invasive disease. Of the possible prognostic factors analyzed, only multiplicity ($p=0.03$) and the number of locations of the tumors ($p=0.03$) were independent prognostic factors in relation to the risk of recurrence. The recurrence free interval was influenced by the therapy. For T1G3 tumors additional intravesical immuno-\chemotherapy or radiotherapy after transurethral resection (TUR) increased the recurrence-free interval significantly.

Conclusions: Because most other parameters did not show additional prognostic value, the T1G3 tumors can be considered as homogeneous concerning the prognosis. Only multiplicity and the number of locations involved added to the prognostic significance of patients with these bladder tumors. In addition we advise to give patients with T1G3 tumors additional treatment after the initial TUR.

INTRODUCTION

The clinical course of superficial bladder tumors (Ta-T1, G1-3, Tis) is characterized by its unpredictability. Although it is obvious that the strategy of treatment differs from muscle-invading bladder cancers, no consensus has been reached about their therapy (1). Transurethral resection (TUR) of all visible tumor remains the cornerstone of treatment, but the initial TUR is followed by a heterogeneous therapy schedule (1,2). Different forms of adjuvant treatment have become available in the last decades. Developments in intravesical chemotherapy and immunotherapy have introduced new treatment options for superficial bladder tumors and allow to individualize therapy (3-5). On the basis of prognostic factors patients with superficial bladder cancer can be divided in subgroups (6,7). Subdivision by tumor stage and grade is most important and can play a role in the treatment selection, the individualization of therapy, and the development of future studies (8,9).

Patients with T1G3 tumors are at special risk, because of the high recurrence rate of these tumors and their special tendency to develop muscle-invasive disease (10,11). The incidence of T1G3 transitional-cell carcinoma of the bladder varies from 6 to 23% of all superficial bladder tumors, recurrences occur in 50 to 90% of patients and progression to muscle-invading disease in 25 to 50% of the patients (6,10,11). Several studies discussed the term "superficial" for these T1G3 patients. Abel showed the unfavourable outcome of patients with pT1 tumors and wanted to reconsider the term "superficial" (12). Jakse et al. defined the 40 T1G3 patients they studied as a high-risk group and asked for special caution (10).

Within the Dutch South Eastern Bladder Cancer Study Group we were able to evaluate the sofar largest series of T1G3 bladder tumors for analysis of prognostic factors, treatment, and recurrence rates.

PATIENTS AND METHODS

From 1983 to 1988, 2,075 patients with newly diagnosed bladder tumors were registered in the Dutch South Eastern Bladder Cancer Study Group. The group is a cooperative organization of 45 urologists (working in 24 hospitals), five radiotherapy

centres, and five pathology departments. 155 Patients with T1G3 tumors were registered according to the TNM classification and grading according to Mostofi, with a slight modification according to Pauwels (13-15). To exclude interindividual variations, one pathologist (E.S) reviewed all slides histopathologically. Of 121 patients the specimens were sufficient for histological characterization, correctly staged and could therefore be included in the study. Of these 104 were men and 17 women, age ranged from 37 to 88 years (median, 70 years). All patients underwent complete resection of the tumor including deep resection of muscle, and random biopsies were taken from 79 patients (65%). Carcinoma in situ was found in 19 of 79 biopsy specimens (24%), and dysplasia in 16 of 79 (20%).

Treatment was instituted according to the preference of the members of the study group, and given immediately after the histological diagnosis. The patients were treated in eight ways, mainly TUR only (48 patients), TUR and radiotherapy (22 patients), and TUR and intravesical immunotherapy or chemotherapy (51 patients). In 16 of the 19 Tis patients adjuvant treatment was given. Five patients underwent a cystectomy after primary diagnosis. The radiotherapy was given as external radiation of 44 Gray on the pelvic region and in addition external radiation of 66 Gray as a boost on the bladder region. The intravesical therapy consisted of mitomycin-c (30mg in 50cc saline), given once a week for one month and thereafter once a month for a total of six months after TUR or BCG (BCG-Tice or BCG-R.I.V.M.), given once a week for six consecutive weeks. Median follow-up for recurrence and survival was 4 years (range, 3-8 years).

Bloodgroup, abnormalities on IVU (visible tumor in the bladder and\or dilatation of the upper urinary tract), tumor configuration, number of tumors, number of tumor locations, cytologic results of voided urine, biopsy results, and mitotic index were analyzed as prognostic factors. The bladder was divided into eleven distinct regions, namely the trigone, right ureteral orifice, left ureteral orifice, right wall, left wall, anterior wall, posterior wall, dome, bladder neck, prostatic urethra and prostate. Malignant cytology was defined as an increase of the nuclear-cytoplasmic ratio, hyperchromatosis of the nuclei, polymorfic nuclei and cells, and the appearance of papillary structures. The mitotic index of the tumor expresses the number of mitotic figures per 10 high power (x40) fields of neoplastic tissue. As criteria for counting mitosis we used the recommendations of Baak (16).

A recurrence was defined as a histologically proven bladder tumor after TURT, progression was defined as the development of muscle-invasive disease.

Statistical methods: recurrence curves were computed with the actuarial method. To test the equality of curves for several groups we used Log-rank tests. We explored the possibility of intergroup differences in important confounding prognostic variables (regarding recurrence) by estimating ß 's in Cox's proportional hazard model. The cut-offs for the various variables were taken with allowance of number of patients in order to obtain statistical significance.

RESULTS

Of the 121 patients with T1G3 bladder tumors, 70 (58%) had recurrent bladder cancer during the follow-up period, and 30 of the 70 (43%) showed progression into muscle-invasive tumors. During the follow-up period, 41 patients (34%) died, 15 of them (36%) from bladder carcinoma. Death due to bladder cancer was not influenced by treatment. Of the patients who died of other causes, two were known to have recurrent superficial bladder cancer at the time of death.

Results of a multivariate analysis of pretreatment prognostic factors are given in table 1.

Prognostic factor		number	β	p-value
age (year)	>70	62	0.15	0.54
	<70	59		
sex	male	104	0.19	0.57
	female	19		
blood group	0+	33	0.21	0.47
	other	66		
IVU	normal	79	0.02	0.93
	abnormal	42		
tumor configuration	papillary	93	0.11	0.72
	solid	27		
tumor number	solitary	74	0.53	0.03
	multiple	45		
number of regions	1	52	0.54	0.03
	>1	69		
cytologic results	malign	39	0.57	0.10
	no	33		
biopsy	no Tis	60	0.14	0.65
	Tis	19		
mitoses (number)	<10	46	0.10	0.68
	>10	70		

Table 1 Multivariate Analysis of Prognostic Factors

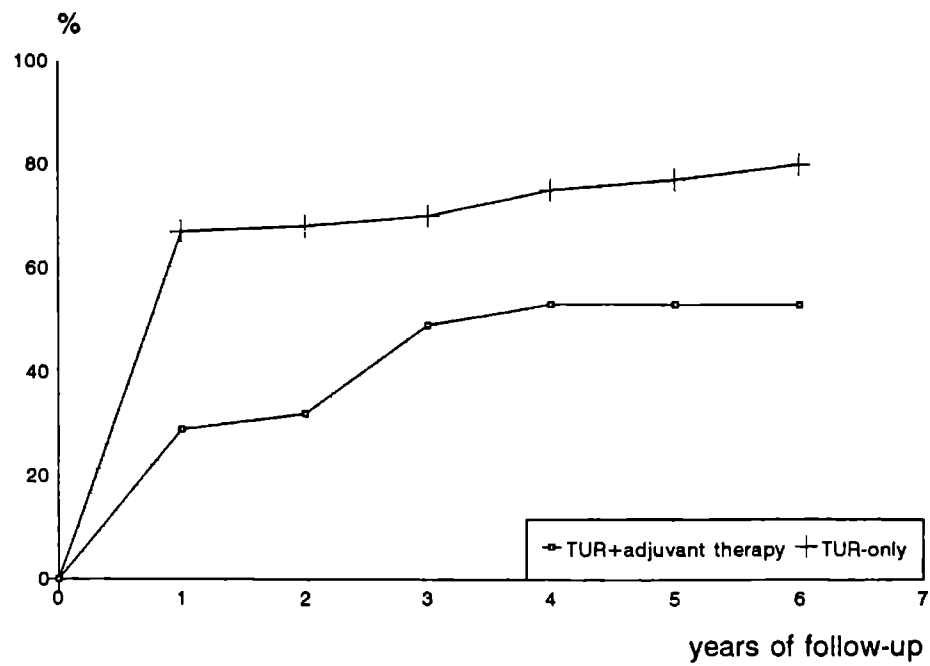
Of the measures examined, multiplicity ($p=0.03$) and location of the tumors in more than one region ($p=0.03$) were both independent prognostic factors for time to recurrence. Especially in patients with multiple tumors located in different regions of the bladder were at risk for recurrence. We did not find additional prognostic significance for bloodgroup, abnormalities on IVU, pretreatment tumor configuration, cytologic results from voided urine, biopsy results and mitotic index ($p>0.05$)

Table 2 compares type of therapy with rates of recurrence and progression. Adjuvant treatment prolonged the time to first recurrence significantly ($p=0.001$) (table 2 and figure 1).

	n	median time of first recurrence (month)	recurrence during follow-up (%)	progression of recurrences (%)
l patients	121	12	58	43
UR only	48	11	75	36
UR+intraves	51	19	55	50
UR+radioth	17	25	35	50
		(p<0.05)	(p<0.05)	(n.s.)

able 2 Treatment and Recurrence, Progression
.B.: -TUR vs TUR+adjuvant treatment: p<O.05 for median time of first recurrence and recurrence during follow-up.
-TUR+intraves vs TUR+radioth: Not significant (n.s.) for median time of first recurrence and recurrence during follow-up.

igure 1: actuarial risk of recurrent disease



Patients with TUR only showed a shorter recurrence-free interval than patients with TUR plus radiotherapy or TUR plus bladder instillations. No significant difference was seen between TUR plus radiotherapy and TUR plus intravesical immunotherapy or chemotherapy ($p=0.34$). We could not find in this study that additional treatment was of use in especially multiple cancers.

Mainly during the first year of follow-up, there was a relatively high actuarial risk of recurrence after TUR alone (64%). The incidence of recurrent progressive disease was not influenced by treatment type ($p=0.74$). It must be noticed that the three treatment arms were completely matched with regard to the other prognostic factors.

DISCUSSION

The prognosis of superficial bladder cancer mainly depends on the risk of recurrent disease. In the majority the recurrences will remain superficial, but about 10 to 15 per cent of the patients will show progression into muscle-invasive disease (6). This cancer has an overall recurrence rate of 50-70 %, while progression into muscle invasive disease occurs in 2-40 % (6).

From several reports on prognostic factor analysis we noticed the clear heterogeneity of the superficial tumors (6,7). It must therefore be questioned why superficial bladder tumors have been classified mainly as a single group, and consequently treatment protocols have been developed and results have been evaluated on that basis (1,3). The most significant prognostic factors in superficial bladder cancer are tumor stage and grade (8,9). Combining these factors a subgroup of T1G3 tumors can be defined. Although considered superficial, this tumor is associated with a high risk of recurrence, and progression, and therefore death. However, the number of T1G3 patients, as a subdivision of superficial bladder tumors, studied in the literature is small (6,7). Several studies underline the high tendency to develop recurrent disease for these tumors (10,11). Although T1G3 transitional-cell carcinomas of the bladder are known to have a worse prognosis than other superficial bladder tumors, consensus about their treatment is lacking. Treatment regimens range from TUR only to radical cystectomy in these tumors.

We were able to study the largest number of patients with T1G3 tumors published sofar. From our study of 121 histologically reviewed T1G3 superficial bladder tumors it

became evident that adjuvant treatment decreases recurrence rate and increases the recurrence-free interval significantly. The prophylactic use of adjuvant intravesical immunotherapy or chemotherapy has been stated to improve treatment results (4,5). Like others we believe that this kind of adjuvant treatment should not be given for every superficial bladder tumor, but it is advisable for T1G3 tumors (1,10,11). Radiotherapy is not widely used for superficial bladder tumors. Although this study was not done in a randomized prospective way, the results indicate the beneficial effect of radiotherapy on recurrence-free interval, as has been stated by others (17,18). Patients with T1G3 bladder tumors need to be treated with special caution. Additive treatment is demanded. A more agressive approach such as cystectomy may be necessary in the long term, especially in patients with a good life expectancy. However, the decision on this radical option may be delayed by intravesical instillations after the initial TUR. Herr et al. concluded in a well documented randomized study that in high-risk patients intravesical BCG can delay disease progression, prolong the period of bladder preservation, and increase the overall survival (19).

Other factors that showed to be of importance were multiplicity of tumors, ureteral obstruction, abnormality in random biopsy and cytology results (7,20-22). In our study, factors such as bloodgroup, abnormalities on IVU, tumor configuration, results of cytology and random biopsy, and mitotic index did not show additional prognostic significance. In our opinion this is because our patients were homogeneous for stage and grade, which are superior indicators for prognosis and could have masked the less important prognostic value of the other characteristics. Only multiplicity and tumor location in different regions of the bladder were independent prognostic factors. With the use of the multivariate analysis only factors that give additional prognostic significance are of value. This may be the reason why our results differ from those of others who studied all different superficial bladder tumor categories. The result of this study that especially patients with multiple tumors located in different regions of the bladder were at risk for recurrence may underline the hypothesis that these patients have diffuse premalignant or malignant changes of the bladder, which are not visible at the time of the initial TURT. This statement could be analyzed with the use of flowcytometry, which showed aneuploid stemcells of normal-appearing mucosa in patients with bladder cancer (23). One would expect that especially patients with multiple tumors benefit from the

additional treatment. However, in this study we were not able to confirm this.

More sophisticated techniques such as flowcytometry, karyometry, and tumor associated antigen expression have been studied recently, but are not yet of clear prognostic value for the daily clinical practice (24-26). They may make it possible to subdivide patients with superficial tumors into individual prognostic categories.

In conclusion, from the results of this study we must advise to give patients with T1G3 bladder tumors additional treatment after the initial TURT. Because of the high recurrence and progression rate, the term "superficial" should be reconsidered or used with caution, with consequences for treatment decision. Patients with T1G3 tumors must be classified and categorized separately. To evaluate these T1G3 tumors together with other superficial tumors does not justify the heterogeneity of these tumors with regard to the risk for recurrence and progression. Additional treatment, especially intravesical immunotherapy and chemotherapy, is advisable to prolong the recurrence-free interval and to improve the survival of patients with T1G3 bladder tumors. Association with bad risk factors, such as multiplicity and location of the tumor(s) in different regions of the bladder, might necessitate a more agressive treatment like cystectomy, especially in patients with a good life expectancy. In developing future treatment protocols the heterogeneity of the "superficial" bladder tumors must be considered. In particular the stage, histologic grade, multiplicity, and the number of locations in the bladder of superficial tumors must play a role in study design. In these studies the optimal treatment for high risk superficial bladder tumors has to be elucidated in prospective randomized protocols, which are now in progress in Europe (EORTC GU group) and in the United States.

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PROGNOSTIC FACTORS IN pT_a-pT_1 SUPERFICIAL BLADDER TUMOURS TREATED WITH INTRAVESICAL INSTILLATIONS

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In a prospective randomized study 387 patients with pT_a-pT_1 papillary carcinoma superficial bladder cancer received, after transurethral resection (TUR), intravesical instillations with immuno- or chemotherapy. A simultaneous computerized analysis of factors predicting recurrence-free interval was performed. All these patients had negative random biopsies. Median follow-up was 27 months (range 12-56 months). During the follow-up period 37.2% of the patients had recurrence and eight patients (2.2%) had progression into muscle invasive disease.

Pretreatment factors analyzed for recurrence were gender, age, history (primary or recurrent disease), location of tumour, number of tumours, pT-stage and grade. After an univariate analysis the number of tumours and location of the tumour in the bladder appeared to be of significant influence on recurrence-free interval. Location of at least one of the tumours in the prostatic urethra, bladder neck, posterior wall, and trigone area was significantly related with a shorter recurrence-free interval. We defined these areas as high risk. Tumour stage showed borderline significance. By using multivariate methods to assess the relative importance of these factors, location of tumour in the high risk region was especially related with a short recurrence-free interval. Only multiplicity added to the prognosis for recurrence. Neither gender, age, history of recurrent disease, size of the largest tumour, tumour stage or grade, gave additional information about risk for recurrence.

We conclude that prognostic factor analysis, as an auxiliary study of trials of patients treated for superficial bladder tumours is mandatory. The prognostic factors related to recurrence-free interval found in this study, e.g. location of the tumour and multiplicity, may be helpful in the stratification neccessary for current protocol design.

INTRODUCTION

Bladder cancer is the third most prevalent malignant disease among male patients and the tenth among female patients in the western world (Feldman, 1986). Approximately 90% of the bladder tumours are transitional cell carcinomas, and 85% are pT_a and pT_1 stages (Silverberg and Lubera, 1987, Soloway, 1989). The disease affects the whole urothelium, and is probably caused by certain genetic characteristics of the patient or by the toxic effects of the urine on the bladder. Consequently, recurrences after resection of the primary tumour are common, making bladder cancer probably the most frequently diagnosed tumour in man (Feldman et al., 1986). These features are at the rationale for the intravesical therapy. Topical application of chemo- or immunotherapy allows contact with tumour-bearing mucosa and consequently may control existing tumours and reduce tumour recurrences (Herr et al., 1987, Soloway, 1989, Kurth et al., 1989). On the other hand we know that not all superficial bladder tumours react similarly to this treatment, because of the varying degree of response to the topical agents (Herr et al., 1989). Therefore it is advisable to individualize the therapy for patients with superficial bladder cancer. Research on prognostic factors may help us to divide these patients into groups who will probably respond to therapy and those who will not. This subdivision is also important for the design of future studies of treatment, especially with respect to the inclusion criteria, follow-up regimens, and for the development of new treatment options, with the aim of improving the prognosis. This is why we performed an auxiliary study on the prognostic factor analysis of this treatment trial, in order to find parameters which are related to chance on recurrence during the follow-up.

PATIENTS AND METHODS

Between April 1987 and December 1990, 469 patients with primary or recurrent resectable pT_a-pT_1 papillary carcinoma or primary carcinoma in situ (CIS) of the urinary bladder, entered the trial which was organized by the Dutch South-Eastern Urological Collaborative Group. Patients were recruited from 27 different urologic centers. The trial is a comparative prospective randomised study of intravesical instillation of three drugs, namely mitomycin-C, BCG-Tice, and BCG-RIVM. The drug was administered after a

complete transurethral resection of all visible tumours (TURT), including deep resection of bladder wall muscle.

Before the tumour resection, random biopsies of normal looking mucosa were taken. Also every area, pale red or hypervascularized, suspected for CIS was biopsied seperately. 82 patients with primary CIS or CIS in the random biopsies were excluded from this analysis. All histopathologic slides were reviewed for stage and grade by a referee pathologist.

Intravesical therapy with mitomycin-C (30mg in 50ml saline) was given once a week for one month and thereafter, once a month for a total of six months after TURT. BCG (BCG-Tice or BCG-RIVM) was given once a week for six consecutive weeks. The choice of therapy was made by means of a restricted randomization. After completing the therapy, control cystoscopy was performed every three months during the first, second and third year.

Pretreatment factors analyzed for prognosis were: gender, age, history (primary or recurrent disease), number of tumours, location of tumour, pT-stage (according to the TMN (IUCC,1987 classification) and grade (according to Koss, 1973). The bladder was divided into eleven distinct areas, namely the trigone, right ureteral orifice, left ureteral orifice, right wall, left wall, anterior wall, posterior wall, dome, bladder neck, prostatic urethra and prostate (figure 1). Location of a tumour in one or more of these areas was used for the assessment of the risk for tumour recurrence.

Time to recurrence was calculated from the beginning of the treatment. Disease-free interval was defined as time interval between the initial TURT and the date of the first positive biopsy. The only endpoint used to assess the prognostic factors was the time until the first recurrence, i.e. the duration of the recurrence-free interval. Patients with no recurrence were analyzed until their last control cystoscopy.

Statistical methods:

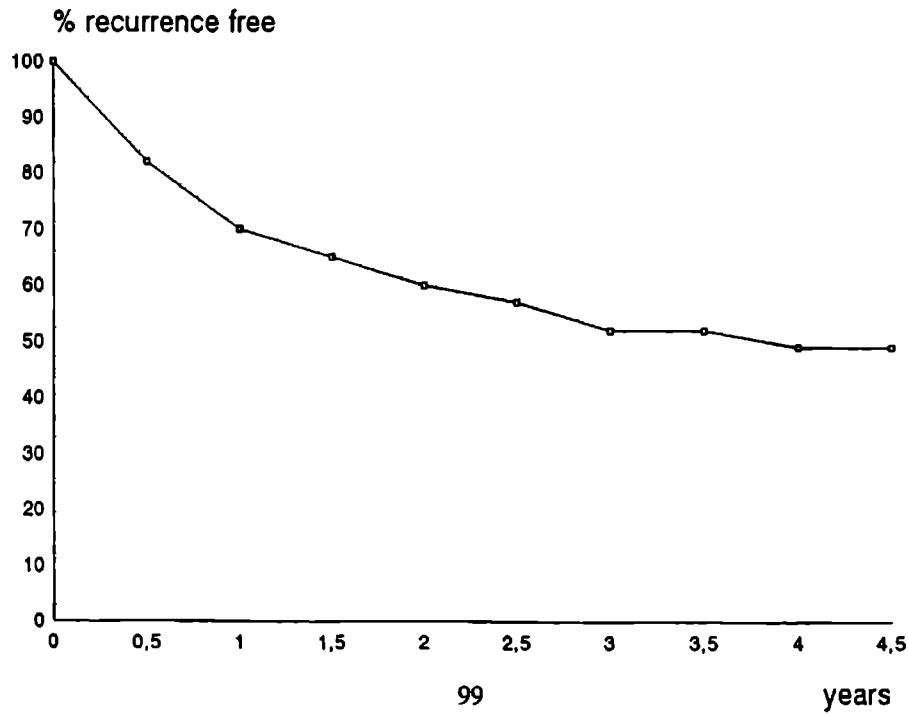
The effects of the potential prognostic factors (co-variates) were analyzed after stratification for treatment. This could be done because no strongly significant differences in recurrence rate and progression to muscle invasive disease was observed among the three treatment arms and because there was a complete match for the prognostic factors. Distribution functions of time to first recurrence were estimated by the method of

Kaplan-Meier. First, an univariate analysis for each factor was performed with the use of the Wilcoxon-Gehan test, secondly we analyzed the factors simultaneously with the stepwise forward selection procedure based on a model-free multivariate logrank test.

RESULTS

Of the 387 patients with pT_a-pT, superficial bladder cancer and negative random biopsies, 371 had sufficient follow-up data. The median age of the patients was 65 years (range 31 to 89 years). 310 patients were male, 61 were female. The median time of follow-up was 27 months (range 12-56 months). During the follow-up period 138 (37.2%) patients had a recurrence. The percentage of recurrence-free patients according to the years of follow-up are shown in figure 1. 25% of the patients showed a recurrence within 41 weeks.

Figure 1: overall recurrence-free interval



In eight patients (2.2%), the recurrent tumour was muscle-invasive. The factors, that were dichotomized for the risk for recurrence, are shown in Table 1. The categories 1 and 2 stand for a better or worse prognosis respectively.

FACTOR	CATEGORY I	CATEGORY II
gender	male	female
age	< 65 years	≥ 65 years
history	primary	recurrent
multiplicity	solitair	multiple
location	other	high risk region
size largest tumour	< 2.0 cm	≥ 2.0 cm
tumourstage	pT_a	pT_1
grade	G_1,G_2	G_3

TABLE 1

Gender and age: We did not obtain a prognostic significance for gender (p=0.35) or age (p=0.10, cut-off point 65 years).

History of recurrence: Because patients with recurrent disease (84 patients) entered the study, we investigated the history of the disease for prognosis. Recurrent disease at entry of the study was of borderline significance for a shorter recurrence-free interval (p=0.12).

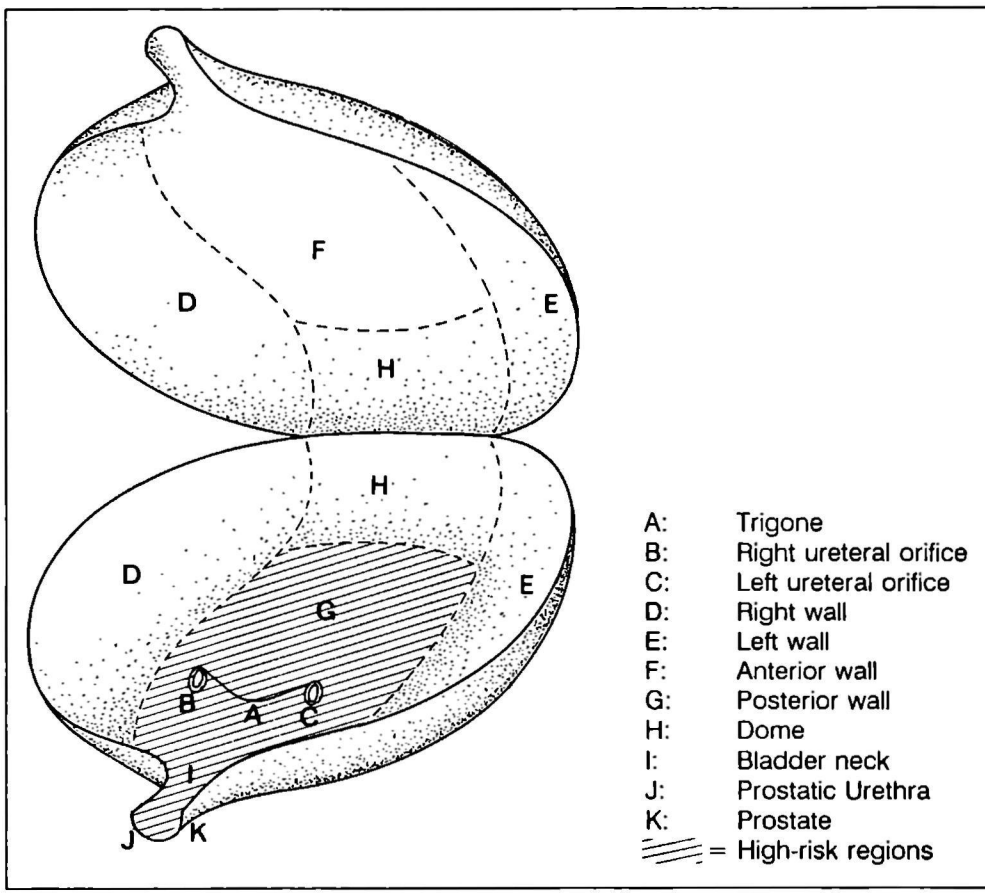
Size of the tumour: Patients were divided into two categories according to size of the tumour. Patients with small tumours (<2.0 cm diameter) did not have a significantly higher risk for recurrence as compared with patients with large tumours (>2.0 cm diameter) (p=0.49).

Multiplicity: The number of tumours resected at presentation was highly significant as a prognostic factor in relation to the recurrence-free interval (p=0.0001). Solitary tumours were more likely not to recur during the follow-up period.

Location of the tumour(s): Because the design of the study included the exact description of the location of the tumour in the different areas (figure 2), we were able to analyze the prognostic impact of the tumour location. In this study, the presence of at least one tumour in the bladder neck, prostatic urethra or the posterior wall (p=0.0001. p=0.0004 and p=0.0008 respectively), was related to a shorter recurrence-free interval. Tumor in the trigone also proved to be of prognostic significance (p=0.02). These areas are

marked in figure 2.

FIGURE 2: high risk regions of the bladder



Tumour stage and grade: The histologic evaluation of the tumour, by a referee pathologist was not a very important prognostic factor in our study. pT_1 tumours, as compared with pT_a tumours, are slightly more frequent ($p=0.07$), and associated with a shorter recurrence-free interval. Grade proved to be of no prognostic significance at all ($p=0.89$).

Table 2 summarizes the results of the univariate prognostic factor analysis.

FACTOR	p-value univariate	stepwise
location	.0001	.0001
multiplicity	.0001	.002
tumourstage	.07	.11
grade	.89	.24
history	.12	.58
size largest tumour	.49	.51
gender	.35	.14
age	.10	.05

TABLE 2

Because different prognostic factors may be interdependent, a study must also consist of a multivariate analysis, in order to define independent prognostic factors. In our stepwise multivariate analysis, the most important prognostic factor appeared to be the location of the tumour ($p=0.0001$). Of all the other factors, which were subsequently evaluated for additional information for the risk for recurrence, only the multiplicity was of additional prognostic relevance ($p=0.002$). The other factors were not independent, so no additional information was obtained by using them for the prognosis of the recurrence-free interval ($p \geq 0.05$).

With the use of the two prognostic factors, location of the tumour and multiplicity we were able to form risk groups. Solitary tumours located in the low-risk region showed a 12 months recurrence-free interval of 88% as compared with 55% of multiple tumors located in the high-risk region. The results are shown in table 3.

			recurrence-free interval (%)		
			6 months	12 months	24 months
LOCATION	NUMBER OF TUMOURS	n			
low-risk	1	98	97	88	83
	2	22	82	72	66
	>2	18	83	61	49
high-risk	1	93	83	71	53
	2	44	93	66	59
	>2	94	71	55	43

TABLE 3

Table 4 shows the percentages of recurrent disease for the different factors and the time to first recurrence. Estimations are given for the period (days) after the initial TURT in which 25% of the patients showed recurrence in a follow-up cystoscopy (R25). Furthermore, figures are provided for the different categories of the estimated proportion of patients who are free of disease after one and two years respectively. We wish to emphasize that when considering the location of the tumours, the R25 values are strikingly different for the high risk and other regions respectively. Similarly, when considering multiplicity, the criteria of solitary and multiple tumours give significantly different R25 values.

FACTOR		n	R25 (days)	proportion disease free 1-year	2-year
location					
	other	138	758	0.82	0.76
	high risk	233	225	0.64	0.51
multiplicity					
	solitary	191	544	0.80	0.70
	multiple	178	202	0.60	0.50
tumour stage					
	pT_a	246	304	0.73	0.64
	pT_1	125	274	0.65	0.52
grade	G_1,G_2	300	290	0.71	0.61
	G_3	71	269	0.68	0.58
gender	male	310	285	0.70	0.62
	female	61	274	0.71	0.50
age	< 65 years	185	274	0.66	0.57
	≥ 65 years	186	363	0.75	0.64
history	primary	287	304	0.72	0.62
	recurrent	84	253	0.65	0.53
size largest tumour					
	< 2.0 cm	133	277	0.71	0.64
	≥ 2.0 cm	211	304	0.72	0.58

TABLE 4

DISCUSSION

A suitable spectrum of incisive prognostic factors is an important tool in clinical oncology. Consequently, one is striving to exploit fully those which are known and to continue searching for new ones. We know that suitable prognostic factors are helpfull in achieving the best treatment regimen in the subsets of patients for which they are meaningful. Prognostic factors also, are helpful in the basic understanding of tumour biology and the design of treatment protocols. No clinical trial studying potential treatment options can do without a prognostic factor analysis.

In this study we found important prognostic factors, relative to recurrence-free interval, tumour location and multiplicity. Tumours located in one of the high risk regions, namely the bladder neck, prostatic urethra, the posterior wall, the trigone and dome, were, after complete TURT, very likely to recur. This point is also mentioned in the report of the British Medical Research Council (UK) subgroup on superficial bladder cancer (Parmar et al., 1989). They performed a follow-up study of a prognostic factors analysis and found that the resection of tumours located in the posterior wall of the bladder, implied a high risk of recurrence. In the study of Parmar and ours it is concluded that certain locations of the tumour in the bladder exhibit particularly high recurrence frequencies. Tumours that arise in these regions should be followed with particular care. It is as yet not known why tumours located in these high risk areas so frequently recur. A possible mechanism might be the contact time with the bladder mucosa and concentration of the intravesical solution in the different regions. Continuous fresh urine is produced by both ureters, consequently rinsing the neighbouring regions (trigone, posterior wall) and preventing the contact of the tumour with the agent installed. The prostatic urethra is normally not in contact with intravesical fluid due to the closed bladder neck. However, the exact role of the instillation schedule and its influence on the recurrence of tumours at these high risk locations in the bladder is as yet unknown and should recieve priority in future studies.

In many investigations the number of tumours at presentation was found to be of importance for prognosis. Parmar and associates stated that the number of tumours found, and the cystoscopy findings performed three months after the initial tumour TUR, were functionally related to the freqency of recurrence (Parmar et al., 1989). Using these two parameters they established relevant prognostic groups of patients, each with a particular appropriate follow-up procedure. Dalesio et al.(1983) found, in an auxilliary study on trials of treatment in superficial bladder cancer performed by the EORTC, that the number of tumours initially resected tumours was a factor influencing recurrence. A similar conclusion had been reached in a large series of 761 patients by Koch et al.(1986). The National Bladder Cancer Collaborative Group A (NBCCA), also obtained comparable results (NBCCA, 1977).

Tumour stage and grade have been widely accepted as prognosticators for the recurrence of tumours (Lutzeyer et al, 1982, Narayama et al, 1983). In groups of patients

with superficial bladder cancer, treated with intravesical instillations of chemo- or immunotherapy, the influence of stage and grade may be less. This treatment may alter the biological behavior of the tumour, especially those at risk for recurrence and progression, and may thus influence prognosis. It is therefore advisable to compare the results of prognostic factor analyses of different studies, only if the treatment mode is identical (e.g. intravesical instillations). In a study of Torrence et al.(1988), who investigated patients treated with intravesical Bacillus Calmette-Guerin, the tumour stage was of little prognostic significance. We reached the same conclusion in this study in which tumour stage was borderline significant for prognosis. Also Parmar et al.(1989) assigned small additional value to tumour stage and grade, as compared with the other more important factors in a multivariate analysis. They mentioned an important disadvantage of histologic typing of bladder tumours, namely the inter-individual variation between the local and referee pathologist, which may be appreciable and consequently decreases the importance of grade and tumour category in a multivariate analysis. Therefore, referee pathology results are essential for the design of a study on treatment modalities. Although all slides in our study were histologically reviewed by one pathologist, the tumour stage and grade were still of little prognostic importance.

In our analysis, history of recurrence at entry, showed only an indication, albeit not significant, for a higher probability of future recurrences. In the study of Dalesio et al.(1983), recurrence rate before entry into the study was a good predictor of prognosis. Also Parmar et al.(1989) found a strong correlation between the recurrence of tumour with the findings at the 3-months cystoscopy. They used these findings as a substitute for a previous recurrence rate.

The other factors examined in this study, age, gender and size of the tumour, did not show prognostic significance. However, they should be included in a multivariate analysis because the groups formed with a prognostic impact, will then be stratified for these factors. Consequently, the results in such a study, obtained with the model-free logrank test, can be adequately used for prognosis. In this way, it is possible to define certain risk groups. Knowing factors such as multiplicity and location of the tumour(s), we may be able to advise on treatment and follow-up strategies. In patients with superficial bladder tumours treated with intravesical instillations, solitary tumours located, for example, in the anterior wall of the bladder, may be followed with less frequent

cystoscopies, because of the good prognosis. On the other hand, multiple tumours located, for example, in the posterior wall of the bladder, should be followed more closely, and may be treated with more intensive intravesical instillation therapies.

Notwithstanding these positive findings, we still need to be more accurate in our estimation of the prognosis of a patient, and therefore it remains imperative to search for other additional parameters. More sophisticated techniques such as flow cytometry and tumour-antigen expression have been studied recently, but are not yet part of daily clinical practice, in spite of their value, for lack of neccesary equipement (Murphy et al., 1986, Blomjous et al., 1988, Falor and Ward-Skinner, 1988, Blasco et al, 1988). With karyometric analysis, these examinations might yield additional information about the likelihood of recurrence, choice of treatment, and survival (van de Poel et al., 1991).

In conclusion, with the use of a prognostic factor analysis, especially the multivariate analysis, we are able to define risk factors for tumour recurrence in patients with superficial bladder cancer, treated with immuno- or chemotherapy. In our auxiliary study of one trial involving 371 patients with pT_a-pT_1 superficial bladder cancer, the location of the tumour in one of the high risk regions and the multiplicity are defined as important prognostic factors relative to the recurrence-free interval. These factors can be used for the appropriate choice of treatment and the determination of follow-up schedules.

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CHAPTER VII

PROGNOSTIC VALUE OF CLINICAL, KARYOMETRIC AND FLOWCYTOMETRIC CHARACTERISTICS IN RENAL CELL CARCINOMA

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The variation in tumor cell differentiation within one renal cell carcinoma, also termed tumor heterogeneity, renders visual tumor grading of these carcinomas difficult. Karyometric analysis enables analysis nuclear characteristics of multiple tumor areas. Hence, karyometric analysis can be used to quantify tumor heterogeneity and thus may aid in a more objective grading of renal cell carcinoma.

Of 121 patients with renal cell carcinoma (RCC) (tumors UICC stages: 1 [5 cases], 2 [23 cases], 3 [33 cases], and 4 [60 cases]) clinical, flowcytometric, and karyometric features were studied to obtain routinely applicable prognostic factors. Only with karyometry several parts of the tumor were analyzed, to obtain a measure of tumor heterogeneity.

The Cox univariate regression analysis showed correlation of several clinical and karyometric characteristics with survival. Flowcytometry did not correlate with survival, but this was probably due to the fact that only one sample of each tumor was investigated. Of the clinical characteristics TNM, tumor size, weight reduction and performance status were significantly related with survival. The karyometric features, especially those measurements associated with tumor heterogeneity (e.g. differences in chromatin texture) were of value in predicting prognosis.

In the Cox multivariate regression analysis the Robson and UICC-stages proved to be the most powerful predictors of survival ($P<0.0001$). Of the clinical features, weight reduction and performance score were the only characteristics offering additional information to tumor stage ($P<0.0001$). From the karyometric analysis quantification of anisokaryosis in the tumor at time of diagnosis offered additional prognostic information. Moreover, the differences of karyometric features within the tumor presumably associated with tumor heterogeneity correlated with survival. Using the features from the multivariate analysis, prognostic groups could be defined.

We conclude that karyometric analysis offers a useful means for quantifying tumor heterogeneity. Multivariate Cox analysis revealed additional value of a grading system based on karyometric analysis to tumor stage. Karyometric analysis can be a useful tool for stratification of patients populations.

Renal cell carcinoma (RCC) accounts for 3 percent of all malignancies and the 5-years survival rate is 30 to 60% (1). The tumor is characterized by its unpredictable clinical course and only limited effective treatment modalities are available. Attempts have been made to classify the patients according to prognostic features. The staging system initiated by Flocks and Kadesky and popularized by Robson and associates is based on the extent of the tumor and correlates with survival (2,3). Another widely used staging model is the TNM system of the American Joint Committee for cancer staging which classifies the tumor according to the anatomical extent of disease (4). This has been the basis of the staging by the International Union against Cancer (5). However, within subgroups of patients as defined by these classification models, the clinical course of RCC is still diverse.

No important improvement in the survival of patients with RCC has been achieved during the last decades. Surgery remains the cornerstone of treatment and the only way to cure the patient. For patients with advanced disease an increasing number of treatment options exists. New treatment modalities like immuno- and chemotherapy are under investigation. They have been developed in an attempt to improve survival (6,7). Because of considerable side-effects of the, mostly palliative drugs the indication for administration must be carefully considered, and only given to those patients who may benefit from the treatment. Individualization in the management of RCC is therefore of utmost importance and further research for prognostic factors is mandatory.

For several tumors, grading offers prognostic information. Considering the extensive tumor heterogeneity in renal cell carcinoma (8) we sought to develop an objective grading system analyzing several parts of the tumor. Karyometry is used to quantify nuclear features and offers an objective measure of tumor phenotypic characteristics. Since heterogeneity will probably result in nuclear differences in light microscopy, comparison of the karyometric feature values of different areas within the tumor can be an objective measure of tumor heterogeneity.

In order to obtain the best possible insight in the prognosis of the individual patient with RCC, we performed a statistical analysis of the most important clinical,

karyometric and flowcytometric features.

MATERIAL AND METHODS

Material

Data were obtained of 121 patients (77 men; 44 women) with a RCC treated between 1983 and 1990 with tumor nephrectomy. The patient age ranged from 32 to 100 years (median 67 years). The median follow up was 21 months (range 4 - 133 months).

All the patients could be classified according to the TNM(V)-model and staged according to the Robson and the International Union against Cancer (UICC) classification models (3,5).

For patients with metastatic disease several adjuvant treatments were given. In 65 M_+ patients one or more forms of immuno- and/or chemotherapy were given (IFNalpha + IFNgamma: n=58, IFNalpha: n=5, vinblastin: n=1, IL2 + IFNalpha: n=1). In 7 patients palliative radiotherapy was given after the tumor nephrectomy. At the time of diagnosis of the primary tumor these additional treatments did not improve survival significantly (log-rank test $P>0.05$). Hence, all 121 patients could be grouped and analyzed for prognostic factors irrespective of the additional treatment given after tumor nephrectomy.

Clinical features

Various clinical characteristics of the patients, in earlier studies found to be associated with the outcome of the disease, were recorded for subsequent analysis. Besides patient age and gender, these included variables used for the classification models like pathologic stage, haematogeneous and lymphogeneous metastases, venal invasion of the tumor and tumor size. Moreover, general indicators of the patients physical condition were documented like performance status (Karnofsky score), history of weight reduction, and serum haemoglobin concentration at time of diagnosis.

Karyometry features

All paraffin-embedded archival material for each patient was reviewed by one pathologist (H.S.). Morphologically different tumor areas were marked for further karyometric analysis. The selection of areas was based on differences in histology (papillary and non-papillary) or cytology (clear, granular, and spindle cell). Karyometric analysis consisted of nuclear morphometry, densitometry, and chromatin pattern analysis with a PC-based image analysis system (VFG-framegrabber board, Imaging Technology, Woburn, MA) in a Compaq 386s personal computer). Image handling and analysis software was written in TIM (TEA, Dordrecht). For analysis, 4 μm thick paraffin-embedded sections were deparaffinized and Feulgen-Schiff stained as room temperature. Within the marked areas nuclei in 50 randomly chosen images were analyzed with a 100x objective. After shading correction and image filtering a local segmentation procedure was performed based on the grey-value histogram in the subimage. Of each nucleus in the recorded images a panel of karyometric features was measured (Table 1).

TABLE 1

- morphometry
  - + AREA (nuclear profile area)
  - + PERI (nuclear profile perimeter)
  - + FELL (elongation factor)
  - + FPE (nuclear roundness factor)
  - + MAXD (maximal nuclear diameter)
  - + Freeman chain code derived shape factors:
    - BEN/NMAC/PASS/DIS/THRES

- densitometry
  - + OD (optical density)
  - + IOD (integrated optical density)
  - + OD CV (coeff. of var. of OD)

- texture analysis
  - + analysis of grey values in maximal diameter axis
  - + Markovian features of co-occurrence matrix:
    - entropy
    - difference moment
    - inverse difference moment
    - rotation moment
    - inverse rotation moment
  - + granulometry (chromatin clots analysis)

Table 1. Karyometric features.

At least 100 tumor nuclei were measured per tumor area. A sequence of 200 images could be analyzed in 5 hours. Of the analyzed nuclei data and images were recorded, thus enabling manual rejection of out-of-focus or faulty segmented objects after the measurement by the operator. For each sample the 2c Deviation Index (2cDI) and 5c Exceeding Rate (5cER) were calculated (9).

Since in all tumors morphologically different tumor areas were marked by the pathologist, a measure of heterogeneity could be calculated as follows. In all tumors for every nuclear feature the tumor area with the highest and lowest value and their differences were determined. We also analyzed whether karyometric analysis of tumor areas marked by the pathologist was different from at random measurements in the tumor. Hence, besides data of the different tumor areas all values of the tumor areas were merged and median and difference of 15th and 85th percentiles were calculated to obtain a subpopulation independent measure of tumor heterogeneity.

The karyometric measurements were tested for inter-individual reproducibility among three technicians. For this purpose we have chosen randomly 28 out of the 121 RCC patients. Three technicians performed independently karyometric measurements of nuclei in the marked tumor areas. The measured feature values were analyzed for their inter-observer agreement using a two-way ANOVA.

Flowcytometric features

After tumor nephrectomy, a sample of approximately 2 cm^3 was cut from the tumor and immediately mechanically or enzymatically processed to tumor suspension. It should be noticed that due to this sampling method, the tumor parts used for flowcytometric analysis were not identical to the areas selected for karyometric analysis. Of the 121 patients, material of 71 patients could be obtained and mechanically processed to tumor cell suspensions and fixed in 70% ethanol. Propidium iodide staining was applied and chicken red blood cells were used as external reference. Several features of the DNA-histogram were calculated: DNA index of G_0G_1-peaks and percentages of cells in different areas of histogram (G_0G_1, G_2M, and S-phase).

Statistical methods

The time from treatment to death was studied using survival analysis. The Kaplan-Meier method was used to estimate the survival function in a group. Differences of survival functions between groups were tested for statistical significance using log-rank test and Wilcoxon test. The 3-years survival rates with 95% confidence intervals were calculated using the Kaplan-Meier method. Cox univariate and multivariate regression analyses (i.e. proportional hazard model) were performed to estimate the influence of the clinical, karyometric, and flowcytometric features on the survival time. Both forward and backward stepwise-selection procedures were used to find the best model in predicting the survival time. The stepwise-selection procedure was performed in two parts. In part I, the selection was used to find all clinical features to define either the best or an equivalent model. The Bonferroni correction was used for model entry of a variable. The same procedure was also performed on the karyometric feature group. In part II the selection procedure was performed on the features from the two feature groups selected in the previous step.

To obtain a prognostic score (RCC-score: good, intermediate, and poor prognosis) based on the selected features in the multivariate analysis, the linear part of the best multivariate model (XBeta) from the selection procedure was used. The levels of the RCC-score were arbitrarily chosen from jumps in the XBeta values of the present patients group.

RESULTS

Clinical features

During the follow-up period 68 (56%) patients died from RCC. The overall 3-years survival was 48%. The Kaplan-Meier estimators for survival for both Robson and UICC classification models in our patients are shown in figure 1 and 2 respectively.

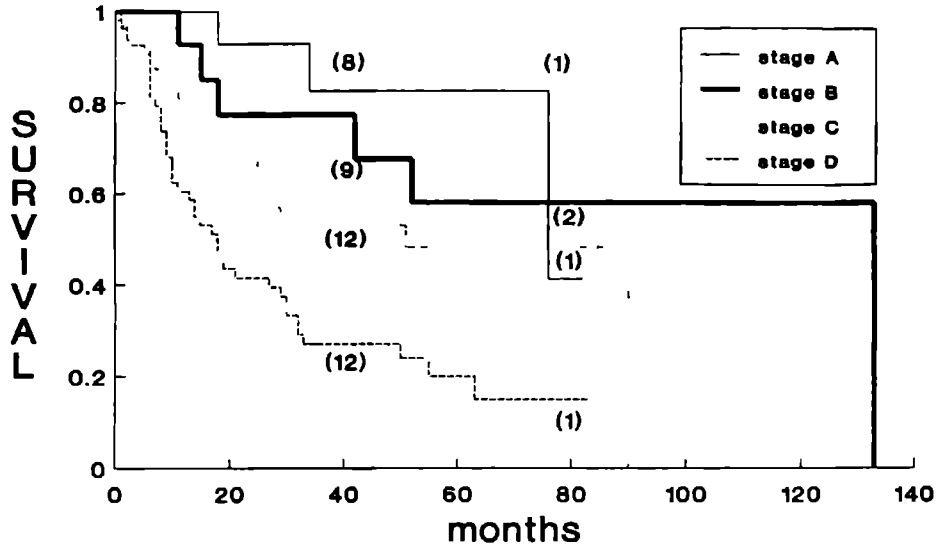

Figure 1. Survival by Robson stage

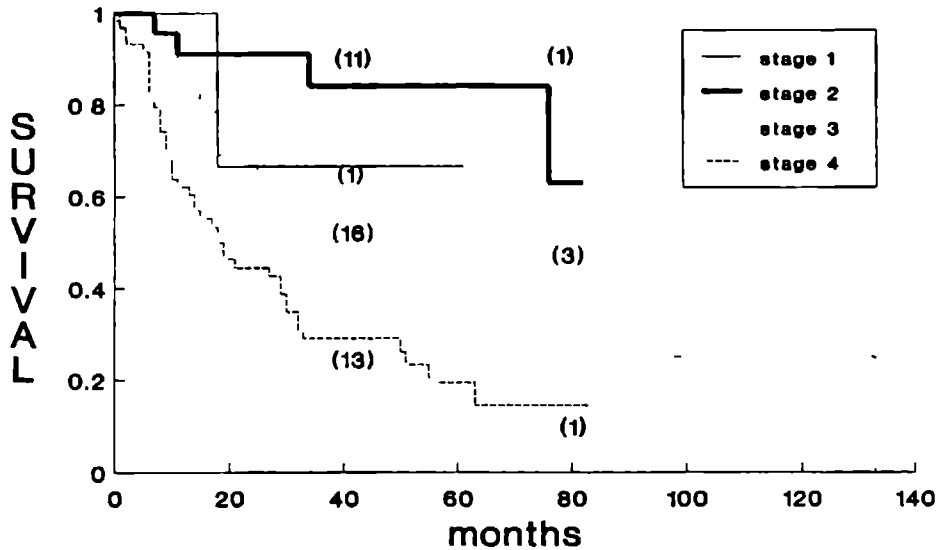

Figure 2. Survival by UICC-stage.

The differences between the 3-years survival percentages of the stages is presented in Table 2.

TABLE 2

	n	3-yrs survival	LCL	UCL
Robson stage				
A	20	0.83	0.60 - 1.00	
B	14	0.77	0.55 - 1.00	
C	32	0.53	0.35 - 0.71	
D	55	0.27	0.15 - 0.39	
UICC stage				
1	5	0.67	0.13 - 1.00	
2	23	0.82	0.67 - 1.00	
3	33	0.59	0.41 - 0.76	
4	60	0.29	0.17 - 0.41	

Table 2. Three-years survival values for different stages in UICC and Robson stages.

Several clinical features appeared to be related with survival. Of the pathological features an increase in T, M, or N stage was significantly correlated with a shorter survival ($P<0.005$, Cox regression analysis). The presence of venal invasion (V), patient age and sex, a low serum haemoglobin concentration at time of diagnosis, and the histological tumor type, however, were not significantly related with a shorter survival ($P>0.10$, Cox regression analysis). Other clinical characteristics that predicted a shorter survival were large tumor volume, a history of weight reduction, and a low Karnofsky score (Table 3).

TABLE 3

	n	β	SE_β	P
UICC-stage (1987)	121	0 8306	0 1948	0.0000
T	121	0 5305	0.1750	0 0024
N	121	0 8488	0 2506	0 0007
M	121	1 2304	0 2666	0 0000
V	121	0 5311	0 2505	0 1206
tumor size	106	0 0682	0 0305	0 0252
tumor type	121	0 0934	0 3334	0.7794
hemoglobine	112	-0 2032	0.1050	0 0531
weight reduction	112	-0 8063	0 2605	0 0020
Karnofsky score	108	-0 0462	0 0134	0 0005
age	121	0 0056	0 0097	0 5615
sex	121	-0 0393	0 2577	0 8788

Table 3. Results from the univariate Cox's regression analysis of the influence of the clinical variables at the beginning of the treatment on the survival in renal cell carcinoma

Karyometry features

The karyometric measurements were tested for inter-individual reproducibility among three technicians. For only three of 32 karyometric features discrepancies were found among the technicians: the same observer scored significantly higher values for one feature (MH3) and differences were found for two other features (MAREA, MIOD). This resulted in a higher percentage of explained variance due to the observers compared to the total variance (2%, 6%, 8% respectively). For the other features the explained variance was less the 1%. Comparison with the percentage explained by the variation between slides showed that differences between observers might be of minor importance: percentages explained by the variation between slides was 85%, 71%, and 68% respectively for the features (MH3, MAREA, MIOD) and 78% to 93% for the remaining features.

The mean number of morphologically different tumor areas as marked by the pathologist per tumor was 2.05 (range 1 to 5) and was not

correlated with survival ($P>0.10$, Cox regression analysis).

Univariate analysis of the karyometric features showed a correlation between several nuclear characteristics and survival (Table 4).

TABLE 4

feature	n	β	SE_β	P
subpopulation-dependent				
L MPASS	115	-0.1449	0.0665	0.0294
L SDOD	115	-8.8587	4.0352	0.0281
U MAREA	115	0.0340	0.0169	0.0436
U SDIOD	115	0.1133	0.0397	0.0043
U MH3	115	5.9251	1.8854	0.0017
D 2cDI	115	0.0609	0.0280	0.0290
D 5cER	115	0.0407	0.0201	0.0427
D MAREA	115	0.0161	0.0081	0.0457
D SDAREA	115	0.0443	0.0160	0.0057
D MFPE	115	10.5628	5.1429	0.0400
D MBEN	115	0.9138	0.4493	0.0420
D SDBEN	115	4.7412	1.6933	0.0051
D SDOD	115	9.9105	3.8077	0.0092
D MIOD	115	0.0583	0.0251	0.0200
D SDIOD	115	0.1117	0.0453	0.0136
D MH3	115	4.9692	2.0552	0.0156
subpopulation-independent				
S IOD	115	0.1369	0.0552	0.0131
MED H4	115	-1.1872	0.5995	0.0477

L	lowest mean population value
U	highest mean population value
D	(D = U - L) diff between values of highest and lowest population
S	diff between 15th and 85th percentile of merged populations
MED	median value of all populations merged
2cDI	= 2c Deviation index
5cER	= 5c Exceeding rate
AREA	= nuclear profile area
BEN	= bending measure difference in maximal and minimal value in smoothed difference Freeman chain code
FPE	= form PE
H3	= Markovian texture feature inverse difference moment
H4	= Markovian texture feature rotation moment
IOD	= integrated optical density
OD	= optical density
PASS	= nuclear shape feature, number of passes through threshold of smoothed difference Freeman chain code

Table 4. Univariate regression analysis according Cox of karyometric features and survival in renal cell carcinoma.

Of the nineteen karyometric features that were related with survival, two were subpopulation independent features. Eleven of the subpopulation dependent features that correlated with survival were features describing differences between tumor areas within the tumor. Karyometric features that showed best correlation with survival were variations in nuclear shape as described by the standard deviation of the bending energy (SDBEN), variances in nuclear size (SDAREA) and 'coarseness' of the chromatin as measured by the inverse difference moment of the requantitated pixel value cooccurrence matrix (Markovian feature H3) (10).

Flowcytometric features

Diploidy was found in 30 samples (47.8%), aneuploidy in 41 (53.2%). Univariate analysis of the flowcytometric features revealed a correlation between the place of the first G_0G_1-peak and survival ($P<0.05$, Cox's regression analysis). The presence of aneuploidy in the tumor was neither significantly correlated with survival nor with tumor stage, the presence of metastases, or venal invasion ($p>0.05$, chi-square test).

Multivariate analysis

Table 5 shows the results of the Cox multivariate regression analysis.

TABLE 5

	ß	$SE_ß$	*P*
Clinical features			
UICC-stage (1987)	0.8748	0.2253	0.0001
Karnofsky score	-0.0398	0.0144	0.0058
weight reduction	-0.7400	0.2803	0.0083
Karyometric features			
D SDAREA (anisokaryosis)	0 0436	0.0184	0.0178

Table 5. Multivariate Cox's regression analysis of clinical and karyometric features with survival of patients with a renal cell carcinoma.

Within this analysis the clinical variables tumor UICC-stage, the presence of weight reduction at time of diagnosis, and the Karnofsky score were the most important characteristics for survival. Besides these clinical characteristics the karyometric feature variation of the standard deviation of the nuclear size in different areas of the tumor showed additional prognostic significance. To study the significance of karyometric analysis in high-stage patients multivariate Cox analysis was performed on all UICC-stage 4 patients with known karyometric analysis (n = 52). Similar to the findings in the entire group, the karyometric analysis offered prognostic value as did the clinical characteristics. However, in the high-stage tumor group the highest value for the karyometric texture feature describing chromatin pattern coarseness per tumor (H3, inverse difference moment) was the best predictor of survival of both the karyometric and clinical features. The presence of weight reduction at diagnosis offered only additional prognostic value to this karyometric feature. This indicates that karyometric analysis was the most important prognostic factor is this group.

With the best predictors for survival, both the clinical (tumor stage, weight reduction, and Karnofsky score) and karyometric features (variation in nuclear size) we were able to form prognostic groups in the entire population of 121 patients. The results are shown in Table 6 and Figure 3.

TABLE 6

XBeta = (0.875 * stage) +
(-0.040 * Karnofsky score) +
(-0 740 * weight reduction) +
(0.044 * D SDAREA)

stage : UICC-stage (1987)
Karnosfky score : performance status (0-100)
weight reduction : weight reduction prior to diagnosis (=1), else 2.
D SDAREA : difference of SD of nuclear size within the tumor.

RCC-score	XBeta	n	3-yrs survival (%)
good prognosis	≤ -2.3	39	80 (65-95)
intermediate prognosis	-2.3 - -1.0	54	41 (27-55)
poor prognosis	> -1.0	28	32 (6-37)

Table 6. Calculation of the RCC-score based on the features found in the Cox's multivariate regression analysis and number of the patients in the prognostic groups based on the RCC-score.

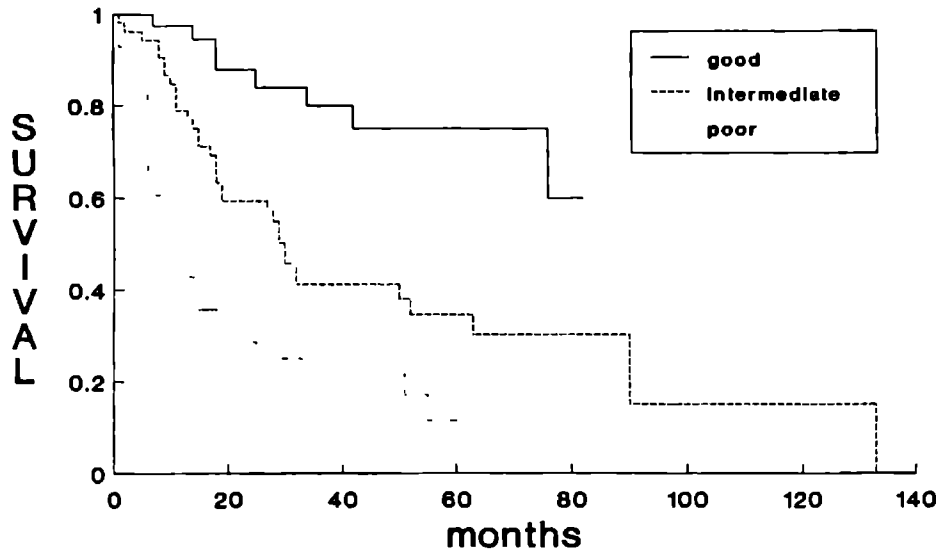

Figure 3. Kaplan-Meier survival curves for the three prognostic groups.

The prognostic score (RCC-score) was calculated using the formula found in the Cox regression analysis. Subdivision of the patients group into three groups based on the XBeta value resulted in significantly different Kaplan-Meier curves (Figure 3). Good prognosis (3-years survival: 80%) was found for patients (n=39) with a XBeta value lower than -2.3, whereas patients (n=28) with a XBeta value higher than -1.0 had a poor prognosis (3-years survival 21%). The 3-years survival for the patients with intermediate prognosis was 41% (n=54).

DISCUSSION

The main problem in the management of patients with RCC is the unpredictable clinical course of the tumor. Since tumor grading of RCC is hampered by considerable tumor heterogeneity we investigated a karyometric grading system for the quantification of differences within the tumor presumably associated with heterogeneity. The karyometric grading was tested for its additional prognostic value to classical and flowcytometric characteristics.

Karyometric analysis, i.e. the quantification of cell nuclear features in light microscopy has been successfully applied for the grading of several cancers. In renal cell carcinoma, Tosi and associates and Bibbo and associates found a positive correlation between morphometric results and survival in patients with stage-I disease (11,12). Murphy and associates described a nuclear shape analysis which allowed the correct assignment of outcome of localized carcinoma which may become available in clinical practice (13). From the studies done so far it has become clear that nuclear size as well as nuclear shape are of prognostic value in RCC. However, the number of patients in these studies was rather small. Moreover, tumor areas for analysis were often chosen at random and none of the studies distinguished different parts within the tumor.

Flow cytometry is known as an easy and reproducible method to determine deoxyribonucleic acid (DNA) content. Otto and associates found a positive relation between the DNA content and risk for recurrence and advised to use this technique in selecting patients for adjuvant chemotherapy after nephrectomy (14). A higher

prognostic significance could be obtained by combining flow cytometry and nuclear grading. Ljungberg and associates suggested that DNA content in RCC might be a superior prognostic indicator than clinical or histological features (15). These results were confirmed by Rainwater and associates in a study with a long follow-up (16). On the other hand Currin and associates recently could not find a significance of flow cytometry as a TNM stage-independent impact on prognosis and re-opened the discussion on the widespread clinical application of ploidy status (17). The ploidy patterns in the different tumor stages in our study were largely in agreement with the findings of Currin and associates We confirmed absence of significant correlation between ploidy and survival. Like Currin and associates, in our study only one sample of the tumor was analyzed. Other studies using multiple samples from the same tumor found a considerable heterogeneity in RCC (14,15,18). Whereas the distribution of diploidy and aneuploidy found in low stage (T_1 and T_2) tumors in our study is similar to earlier findings, the number of aneuploid high-stage tumors is relatively low (59%) and may illustrate the inaccuracy of only one sample as taken in these larger tumors. Tumor heterogeneity in the larger tumors is most likely the explanation for the lack of correlation of ploidy with survival.

From these flowcytometric studies we learned that considerable heterogeneity of tumor cell populations exists in RCC. The origin of multiple cell populations within the same tumor can most likely be found in genetic instability, a trait of aggressive neoplasms. Karyometric analysis offers a means to quantify nuclear features within the cell clones. Hence, the technique can be used to obtain a measure for the level of phenotypical differences between the cell clones within the same tumor. We postulated that the degree of nuclear phenotypic differences within the tumor as assessed by karyometric analysis is indicative for tumor heterogeneity and thus may in fact correlate with genetic instability in the tumor development. Since increased genetic instability might result in more rapid progression to malignant, e.g. metastasizing phenotype the karyometric grading of tumor heterogeneity might be a good predictor of tumor malignancy.

In the present study morphologically different areas within the tumor were selected by the pathologist and karyometrically analyzed. Whereas the number of marked tumor areas per tumor was of no prognostic value, data from our study

indicate prognostic value for the degree of heterogeneity as assessed by the differences of karyometric phenotype between subpopulations. Heterogeneity in DNA content (2cDI), nuclear size, shape, and chromatin pattern were all correlated with survival, which is in agreement with other studies (11,12,13).

In tumors already metastasized at time of diagnosis (stage IV) karyometric analysis appeared to be the best predictor of survival. However, the karyometric features describing heterogeneity in the tumor were less predictive of survival in this patient group. It is therefore tempting to speculate that in metastasized disease malignant behaviour of the tumor is more determined by the presence of malignant cell clones rather than by the chance of development of an even more malignant phenotype. In this light, we consider high-stage tumor as completely dedifferentiated cancer that gained full malignant potential.

To evaluate the additional value of karyometric tumor grading and flowcytometry to classical tumor characteristics we also analyzed several clinical characteristics.

The stage of the tumor, including invasion of the vena cava, and the presence of lymph nodes or metastases allow to make subdivisions of patients with regard to survival. The local extent of tumor at the time of surgery is the most important single variable in determining survival (19). Because the TNM(V) classification explicitly defines the anatomic extent, it is often at the basis of further stratification of risk factors (19,20,21). The patients in the current study were classified according to the TNMV and Robson system and it appeared that a higher stage was related to a lower survival. More explicitly, not only tumor stage but also the presence of lymph node or distant metastases showed to be an important prognostic indicator for survival. In the univariate analysis they all appeared to be of prognostic significance. It is now clear that vena cava invasion, when curatively operated, is not a prognostic indicator as such (22). The reason is that it is often associated with factors that heavily influence survival, as was also seen in our study (20).

Other features, related to survival, were performance status (Karnofsky score) and a history of weight reduction. These obvious clinical characteristics were also found by others, and should be included in a prognostic factor analysis (19,21). Size of the primary lesion, indirectly related to the T-stage, was not an independent

prognostic factor, as was also seen by others (21). RCC are divided in the literature into clear cell, granular cell, spindle cell, and oncocytoma type tumors. Although oncocytomas are characterized by a favourable clinical course, the prognostic importance of the other tumor types is not clear (22-26). In our series oncocytomas were excluded and no correlation with survival was found for the other tumor types.

To obtain features that independently predict survival, a multivariate analysis was performed. It was obvious that tumor stage, history of weight reduction, and the karyometric features that illustrate the heterogeneity of the tumor appeared to be the most important prognostic factors in patients with RCC. With these factors we were able to define certain risk groups that can be used for treatment decision and the development of future clinical trials.

We conclude that clinical and karyometric features correlate with tumor behaviour. Moreover, a combination of karyometry and clinical data offers the best prediction of survival. Tumor heterogeneity, as quantificated by karyometric analysis plays an important role in tumor behaviour. The factors analyzed in this study may be of use to individualize the treatment of patients with RCC.

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CHAPTER VIII

PREDICTING TUMOR PROGRESSION OF CLINICALLY LOCALIZED ($T_{1-3}N_0M_0$) RENAL-CELL CARCINOMA AFTER RADICAL NEPHRECTOMY

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SUMMARY

The risk of local recurrence or development of distant metastases in patients with a locally confined ($T_{1-3}N_0M_0$) renal cell carcinoma (RCC) is 45-65%, and additional treatment is warranted to improve survival rates. Adjuvant immunotherapy might be beneficial to kill micro-metastases that exist at the time of surgery. Because this kind of treatment has considerable side-effects, selection of patients based on prognostic factors is mandatory.

Material from 52 patients with $T_{1-3}N_0M_0$ tumors was studied among a population of 121 patients with all stages of RCC. During follow up of at least 3 years, 21 patients developed local recurrence or distant metastases.

In an univariate analysis for risk of progression, of the clinical (age, sex, weightloss, performance status, and serum hemoglobin concentration) and pathological characteristics (T stage, tumor size, and tumor type), only performance status appeared to have prognostic significance; the karyometric characteristics of nuclear shape, nuclear size, and chromatin patterns were also predictors of tumor progression. In a multivariate analysis differences in chromatin pattern were the best predictors of survival; only T stage added to the prognostic significance.

We conclude that karyometric analysis is a powerful tool in predicting tumor progression in patients with clinically localized RCC. Tumor heterogeneity expressed as different nuclear chromatin patterns was the most powerful indicator of progression. Those findings are particularly valuable in stratifying RCC patients for follow up shedules or even adjuvant treatments.

Renal-cell carcinoma (RCC) accounts for 2-3% of human malignancies. The overall 5-year survival rate is 30-60% [1]. In 50% of patients, the carcinoma is confined to the kidney. For these patients radical surgery offers the only chance of cure. The 5-year survival rate of these patients ranges from 50 to 90 % [2-5]. Therefore, unless the tumor seems to be susceptible to radical surgery, the outcome remains unpredictable and a substantial number of patients will die from RCC. The deaths will probably be due to outgrowth of micro-metastases that cannot be detected by radiologic examinations, but already exist at the time of tumor nephrectomy.

The rationale of adjuvant chemotherapy or immunotherapy after tumor nephrectomy in low-stage RCC is based on the use of animal models. In animal models, immunotherapy has been effective, especially in microscopic disease more than in macroscopic disease [6,7]. However, in humans the situation is not so clear. In patients with colon carcinoma with only regional nodal involvement, adjuvant chemotherapy has improved survival rates [7]. In patients with localized RCC, adjuvant immunotherapy or chemotherapy might improve the outcome of the disease after tumor nephrectomy. Immunotherapy can have serious side effects, so it must be carefully considered and administered only to specially selected patients. Knowledge of factors that can be used to predict tumor recurrence or progression into metastatic disease is essential in the selection of appropriate patients.

Clinical and histopathologic characteristics have been studied, and prognosis has been shown to correlate well with performance status, tumor stage, and tumor grade [3-5]. In view of the low reproducibility of tumor grading, quantitative light microscopic techniques with image analysis have been used to describe histological tumor features [8]. Several karyometric features have been found to correlate with prognosis [8-11].

We have investigated the use of clinical, pathologic and karyometric features of patients with clinically localized RCC for the prediction of local recurrence or the development of metastatic disease.

Patients

Of a total of 121 patients, we analyzed data on 52 patients (31 men; 21 women) with clinically localized RCC treated between 1983 and 1989 with radical nephrectomy. Their median age was 57.4 years (range, 29-93 years). The followup period was at least 3 years (median, 60 months).

Radiographic staging was done with computed tomography of the abdomen and tomography of the lungs. No evidence of lymph node metastases was found in pathologic examination of lymphadenectomy specimens. The patients could be classified according to the tumor-nodes-metastases (TNM) system developed by the International Union against Cancer, which classifies cancer cases according to the anatomic extent of disease [12]. Localized disease was defined as stage $T_{1-3}N_0M_0$. This means that all patients potentially could be operated on curatively.

Clinical features

Various clinical features that had earlier been found to be associated with the outcome of the disease were recorded. Besides age and sex, they included performance status (Karnofsky score) and a history of weightloss. Serum hemoglobin concentration was also tested. Of the pathologic characteristics, T stage, tumor size, and histologic tumor type were recorded.

Karyometric features

All paraffin-embedded material was reviewed by one pathologist (H.E.S.). Morphologically different tumor areas were marked for karyometric analysis. The selection of areas was based on differences in histology (papillary and non-papillary) or cytology (clear, granular, and spindle cell). Karyometric analysis consisted of nuclear morphometry, densitometry, and chromatin pattern analysis with a PC-based image-analysis system (VFG-framegrabber board, Imaging Technology, Woburn, MA) in a Compaq 386s personal computer). Image handling and analyisis software was written in TIM (TEA, Dordrecht). For analysis, 4-μm-thick paraffin-embedded sections were deparaffinized and Feulgen-Schiff stained at room temperature. In

figure 1 an example is given of a light-microscopic image that is processedin steps to the image used for karyometric analysis. After shading correction and image filtering a local segmentation procedure was performed based on the grey-value histogram in the subimage.

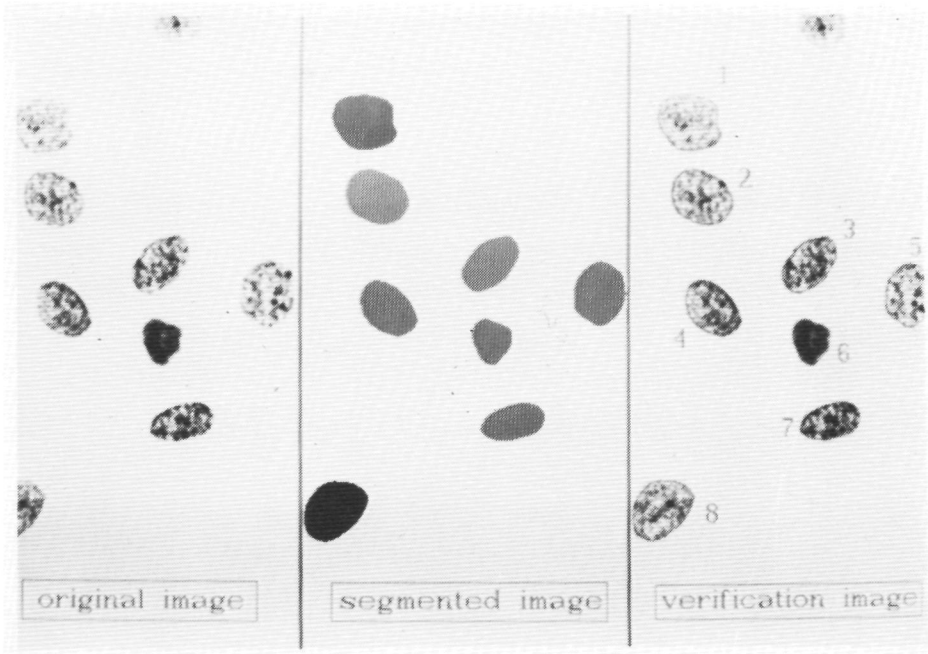

Figure 1. Summarize of the image processing steps:
A. original black and white image
B. tresholded and segmented image
C. verification image for selection of nuclei of interest after karyometric analysis

Because the pathologist marked in each tumor morphologically different tumor areas, heterogeneity could be calculated as follows. In all tumors for every nuclear feature the tumor area with the highest and lowest value and their differences was determined. We also analyzed the benefit of karyometric analysis of different tumor areas marked by the pathologist compared to at random measurements in the

tumor. Hence, besides data of different tumor areas all values of the tumor areas were merged and median and difference of 15th and 85th percentiles were calculated to obtain a subpopulation independent measure of tumor heterogeneity.

The karyometric measurements were tested for inter-individual reproducibility among three technicians. The technicians performed independently karyometric measurements of nuclei in the marked areas. The measured feature values were analyzed for their inter-observer agreement using two-way ANOVA [13].

Statistical methods

The time from tumor nephrectomy to tumor progression was studied. Progressive disease was defined as renal fossa recurrence or development of distant metastases. The Kaplan-Meier method was used to estimate the progression rate. Differences obtained were tested for statistical significance with the log-rank test and the Wilcoxon test. The 3-year tumor progression rates, with 95% confidence intervals, were calculated with the Kaplan-Meier method.

Cox's univariate and multivariate regression analyses (i.e., proportional hazard model) were performed to estimate the influence of the clinical and karyometric features on time to tumor progression. Both forward and backward stepwise selection procedures were used to find the best model for predicting the disease-free interval. The selection procedure was performed in two parts. In part I, the selection was used to find all clinical variables to define either the best or an equivalent model. The Bonferroni correction was used for model entry of a variable. The same procedure was also performed on the karyometric feature groups. In part II we used the selection procedure on both clinical and karyometric features selected in the previous step.

RESULTS

Clinical features

During the followup period 21 (40%) patients developed progressive disease; 15 (71%) of the 21 died of RCC. The 3-year progression rate was 33% for T_1 (n=5),

29% for T_2 (n=23), and 47% for T_3 (n=24). The differences between the progression rates were not significant in the Wilcoxon test ($P = 0.19$) or the log-rank test ($P = 0.17$).

In the univariate Cox's regression analysis of the clinical features, only the patients with low Karnofsky scores at the time of diagnosis were at risk to develop tumor progression, and the relation was significant ($P < 0.05$). Tumor stage, patient age and sex, serum hemoglobin concentration at the time of diagnosis, histologic tumor type, tumor size, and history of weight loss, were not significantly related to progression ($P>0.10$). The results of the univariate analysis are shown in Table 1.

TABLE 1

	Beta	SBeta	P	R
Sex	0.674	0.450	0.1343	0.041
Age	-0.0079	0.016	0.6241	0.000
Karnofsky score	-0.0508	0.243	0.0369	-0.137
Weight loss	-0.7720	0.487	0.1130	-0.064
Hemoglobin	-0.100	0.178	0.5722	0.000
T stage	0.686	0.408	0.0927	0.076
Tumor size	0.094	0.071	0.1836	0.000
Tumor type	0.350	0.520	0.5006	0.000

Table 1. Univariate Cox's regression analysis of clinical features and tumor progression.

Karyometry features

The karyometric measurements were tested for inter-individual reproducibility among three technicians. For only three of 32 karyometric features discrepancies were found among the technicians: the same observer scored significantly higher values for one feature (MH3) and differences were found for two other features (MAREA, MIOS). This resulted in a higher percentage of explained variance due to the observers compared to the total variance (2%, 6%, 8% respectively). For the other features the explained variance was less than 1%. Comparison with the percentage explained by the variation between slides showed that differences between observers

might be of minor importance: percentages explained by the variation between slides was 85%, 71%, and 68% respectively for the features (MH3, MAREA, MIOD) and 78% to 93% for the remaining features.

The number of morphologically different areas per tumor was not correlated with tumor progression ($P>0.10$). Of the nine karyometric features that were related to progressive disease, only one was an area-independent feature. Differences in karyometric-feature values between selected tumor areas were highly correlated with progression. Univariate analysis of the karyometric features showed a correlation between several nuclear characteristics and progression (Table 2).

TABLE 2

	Beta	SBeta	P	R
L 2cDI	-0 234	0 117	0 0464	0 118
U MBEN	2 243	1 108	0 0429	0 122
D SDAREA	0 102	0 041	0 014	0 170
D MSCAT	1 281	0 454	0 0048	0 206
D MH1	5 934	2 009	0 0032	0 219
D SDH1	6 322	2 424	0 0091	0 185
D MH3	60 002	19 386	0 0020	0 232
D MH5	2 799	1 001	0 0051	0 204
MEDPASS	0 254	0 121	0 0351	-0 132

Note L= lowest population value
U= highest population value
D= U-L
MED= median value for entire tumor (all populations merged)
2cDI= 2c Deviation Index (Böcking,1984)
MBEN= mean bending nuclear shape factor (based on smoothed differences in Freeman chain code), describing nuclear irregularity
SDAREA= standard deviation of nuclear area
MSCAT= mean scatter variance in pixel values (texture feature)
MH1= mean H1 (Markovian feature entropy)
SDH1= standarddeviation H1
MH3= mean H3 (Markovian feature inverse difference moment)
MH5= mean H5 (Markovian feature inverse rotation moment)
MEDPASS= median value in tumor of PASS (nuclear shape factor based on smoothed differences in Freeman chain code)

Table 2 Karyometric features significantly correlated with tumor progression in univariate Cox regression analysis

Multivariate analysis

Table 3 shows the results of the multivariate Cox regression analysis. Several karyometric features could be entered in the model.

TABLE 3

Step	Beta	SBeta	P
1. D MH3*	61.330	19.90	0.002
2. Tumor stage	1.086	0.492	0.027

* Differences between populations with highest and lowest values of the inverse difference moment of the co-occurence matrix (Markovian texture feature) within tumor.

Table 3. Multivariate Cox regression analysis of clinical and karyometric features and tumor progression.

A most significant result was found for the differences in chromatin pattern between tumor subpopulations, as described by the inverse difference moment of the co-occurence matrix (Markovian texture feature MH3). Otherwise, only T stage showed additional prognostic significance. In the multivariate analysis, sex, age, presence of weightloss at the time of diagnosis, histologic tumor type, tumor size, and Karnofsky score did not add to prognostic significance.

DISCUSSION

The chance of local recurrence or development of distant metastases in patients with locally confined RCC (T_{1-3} N_0 M_0) is 45-65% [11,14]. Moreover, the prognosis of patients with progressive disease after radical tumor nephrectomy for a locally confined tumor is very poor: a mortality rate of 74% at 1 year has been recorded [14].

Many clinical trials have studied the role of immunotherapy in patients with RCC [15]. In patients with metastatic disease, 14-30 % reacted positively to this type of therapy [16,17]. Especially patients with good performance status, relatively low tumor burden, no central nervous system or bone metastases, and a long interval between

nephrectomy and appearance of metastases seemed to benefit from immunotherapy [8,19]. Because patients with a low tumor burden react better to immunotherapy, patients with localized disease might benefit from this kind of treatment. In these patients early detection of progression is neccessary. However, no applicable technique has been developed for the detection of micrometastases or remaining tumor cells in the renal fossa that can be used to predict the chance of tumor growth after radical nephrectomy for locally confined RCC. To identify patients at risk a prognostic factor analysis is mandatory. In the present study, we investigated the use of clinical, pathologic, and karyometric features to predict tumor progression of locally confined RCC.

Because, for decades, the surgical approach to RCC has been the mainstay in treatment, the histologic results and material have been applied to determine prognosis [20]. Factors described in the literature are tumor-cell type, nuclear grade, mitotic rate, tumor heterogeneity, and cytologic features [21-27]. More recently, karyometric analysis has been shown to be of prognostic significance. Several authors found a positive correlation between morphometric results and survival in patients with stage I disease [9,10]. Others found a prognostic discriminant in the nuclear size of all RCCs [28]. Murphy and associates described a nuclear-shape analysis that predicts outcome of a localized carcinoma and might become available for clinical use [11]. Therefore nuclear size and shape are of prognostic value in RCC.

Flow cytometric studies have shown considerable heterogeneity of tumor-cell subpopulations in RCC [29,30]. We tried to develop a routinely applicable method of quantitative tumor grading, taking tumor heterogeneity into account. Morphologically different tumor areas were marked and karyometrically analyzed. The number of marked tumor areas per tumor was of no prognostic value, but the karyometric data in the different areas had predictive value for tumor recurrence. Like Murphy et al. [11], we found a shorter time to tumor progression or local recurrence in patients who had tumor-cell populations with irregularly shaped nuclei. Unlike Tosi et al. [9], we found absolute nuclear size to have no predictive value. However, the presence of heterogeneity in nuclear size (anisokaryosis) in the different tumor areas was significantly related to tumor progression. The importance of heterogeneity is also illustrated by the results of the chromatin-texture analyses: recurrence or progression

is earlier in patients with a high variance in nuclear chromatin patterns as measured by the Markovian texture features.

Although tumor heterogeneity has been described for several tumors and its correlation with prognosis has been reported, we can only speculate on the reason for the worse prognosis of heterogeneous tumors. Most likely, the genetic instability of the tumor, reflected by heterogeneity, is the reason. Hence, karyometric analysis, which quantifies the nuclear phenotypic characteristics, not only describes the extent of nuclear atypia in the tumor but also provides information on tumor biology. The presence of large differences in phenotype between populations, as described by karyometry, could be a useful measure of genetic instability. Presumably, the chance of development of a high-grade malignant phenotype increases in heterogeneous tumors; this would account for the prognostic value of karyometric analysis.

To obtain features that predict recurrent disease independently, we performed a multivariate analysis. Of the clinical and karyometric characteristics, the features describing variance in chromatin pattern within a tumor were significantly correlated with the chance of local recurrence or of development of metastatic disease in the multivariate model. Of all the other clinical features, only tumor stage yielded additional information.

We therefore emphasize that the features of karyometric analysis can be used to predict the outcome of patients with localized RCC. In patients with high anisokaryosis, irregularly shaped nuclei, but above all variance in chromatin pattern, there is a considerable chance of progression. These patients must be followed more closely or even treated with adjuvant immunotherapy from the onset after radical nephrectomy. In a recently activated randomized phase-III study that uses adjuvant subcutaneous recombinant interleukin-2 versus no treatment in patients with RCC and no evidence of disease after tumor nephrectomy and lymphadenectomy, we will include the karyometric characteristics to evaluate their prognostic significance in a prospective study and to individualize patients followup schedules.

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CHAPTER IX

SUMMARY AND PROSPECTS

1. Summary

In Chapter I.1. the rationale of research on prognostic factors in urological oncology has been discussed and it is concluded that prognostic factors can be clearly defined and that a 'prognostic stratification' forms the basis of the design of treatment protocols. In this context it is concluded that the contribution of the statistician is essential, and it is imperative that the statistical analysis have to be performed in an unbiased manner. In Chapter I.2. an overview is given of the prognostic factors in germ-cell tumors. Several histological classifications and staging models are described and their importance for treatment decision and prognosis is highlighted. Using these characteristics in combination with biochemical parameters, we are able to define certain risk groups. These results are at the basis for further research on prognostic factors in these tumors. In Chapter I.3. a summary is given of the state of the art on prognostic factors in metastatic prostate cancer. Particularly the biochemical markers are of interest for the prognosis and in monitoring progression. These factors are, in addition to the histological and radiographic parameters, also meaningful for the prognosis and for the individualization of treatment. In Chapter I.4. the prognostic factors of patients with superficial bladder cancer are discussed. A combination of clinical and pathologic characteristics is useful to predict the likelihood of recurrence. Because new treatment options have been developed, the value of these parameters is becoming increasingly important. In Chapter I.5. a summary of the prognostic factors in renal-cell carcinoma is given. With the clinical, pathological, and biochemical parameters already known, the course of this cancer may not be predicted. Most of the sofar known parameters are summarized here. Recent progress on cytogenic and karyometric characteristics of tumor cells as to prognosis predictors is also discussed.

The results of the review of prognostic factors as given in chapter I, formed the impetus for the investigations carried out for this thesis. Based on the knowledge of these prognostic factors, new investigations were initiated with the aim to define better the prognosis for a patient with an urological tumor and judiciously to select the appropriate treatment and follow up schedules.

In Chapter II an investigation is done of the value of the classification models of the European Organization for Research and Treatment of Cancer (EORTC) and of the

Indiana (US) group for patients with disseminated germ-cell tumors. Both models were useful for predicting the survival of patients treated with induction chemotherapy. As an additional prognostic parameter, the size of the retroperitoneal tumor before and after chemotherapy appeared to be of importance. Radiographic changes in the size of the retroperitoneal tumor during chemotherapy appeared to be of considerable prognostic significance with regard to the histology of the tissue resected after chemotherapy and therefore for prognosis. With this knowledge, further individualization of the treatment for these patients may be designed.

In **Chapter III** an update is given of prognostic factors in disseminated prostate cancer. This side-study of a well-documented trial of hormonal treatment showed that using performance status, haemoglobin and alkaline phophatase as group criteria, one may define the corresponding risk groups. Especially for the high-risk group, new treatment modalities may be useful to improve the prognosis.

In **Chapter IV** an investigation is done of the exact value of the biochemical markers, alkaline phosphatase, prostate acid phosphatase and prostate-specific antigen in monitoring the hormonally treated prostate cancer patient. Especially the PSA was useful for this purpose and may permit the omission of routine bone scanning. This procedure is now being adopted in new trials.

In **Chapter V** a controversial subgroup of patients with superfical bladder cancer has been examined. The results of the treatment of patients with pT_1G_3 bladder tumors, gathered by a coöperative group of urologists in the south-eastern part of The Netherlands, were evaluated retrospectively. None of the prognostic factors examined, appeared to be of additional prognostic value in these selected patients. It was concluded from this study that additional treatment with intravesical immuno-, or chemotherapy, or radiotherapy, after the initial transurethral resection is mandatory, to improve survival.

In **Chapter VI** a side-study on prognostic factors is performed in patients with superficial bladder tumors treated with intravesical instillations. In this well documented trial, multiplicity and the location of the tumor were of prognostic significance for the recurrence-free interval. Especially location of at least one tumor in the regions trigone and posterior wall of the bladder were an additional risk factor for recurrence after the first initial intravesical treatment. It was concluded that especially these patients should be followed more closely.

In Chapter VII a prognostic factor analysis of clinical, flowcytometric and karyometric characteristics in renal-cell carcinoma is performed. In this heterogeneous group of all stages of renal-cell carcinomas it was obvious that from among the clinical factors only performance status and a history of weight loss were of prognostic importance. Those karyometric parameters which characterize the heterogeneity of the tumor, were also of importance for the prognosis for patients with renal-cell carcinoma. This heterogeneity may imply biological instability of the tumor and thus related to a shorter survival of the patient. The flowcytometric characteristics were not of additional prognostic significance, but this may be caused by the fact that only one sample of the tumor was studied. The aforementioned heterogeneity was thus not available for analysis.

In Chapter VIII a subgroup of $T_{1-3}N_0M_0$ renal-cell carcinoma was subject of investigation. In particular it confirmed the value of the karyometric characteristics; the karyometric characteristics which stand for heterogeneity were of utmost importance. In this context karyometry informs us on the malignant potential of the tumor and may help us to select patients for additional treatment, for instance immunotherapy.

2. Prospects

"Is this the future or is the future already present?"

Prognostic factor analysis in urological cancer will continue to play an important role in the future. With the number of treatment options on the increase, their evaluation will need exact prognostic factors. Only in this way we will be able to improve our treatment results for carcinoma patients. A phase III randomized trial on treatment should also be accompanied by a so-called side-study of prognostic factors. These well-documented trials gives one the opportunity to identify the exact values of prognostic factors in relatively homogeneous groups of patients. In order to be able to draw meaningful conclusions it is often necessary to recruit a large number of patients in a relatively short period of time. Therefore, special effort must be made to develop large international multicentre trials. This gives additional merit to international organisations like the European Organization for Research and Treatment of Cancer (EORTC). The value of a prognostic factor analysis is also increased by these large number of patients, treated within a short period of time, and answering the same inclusion and exclusion

criteria. Only in this way, an accurate evaluation of the treatment for the individual patient is guaranteed.

In order to make progress in urological oncology, it is necessary to continue the research on prognostic factors. The value of prognostic factors must be defined for the various levels of cancer and the patient whom it affects. In the field of the basic research, cytometric, cytogenic and molecular abnormalities must be more extensively investigated and may, in the future be included in a routine clinical setting.

Germ-cell tumors

For disseminated germ-cell tumors, timing and extent of treatment is still investigational. New prognostic factor analysis of large series of patients will better define risk groups (Mead et al). Especially patients who have a high probability of failing treatment will be included in new experimental studies. Because these very intensive chemotherapy schedules still have severe and potentially lethal side effects, the research on prognostic factors, indicating these poor risk patients is vital.

For this rare tumor, it is mandatory to develop large multi-centre studies in order to improve the relatively good prognosis. Especially further individualization of treatment; e.g. the number of cycles of chemotherapy or the exact indication for lymph node surgery, must be the goal. Also a better monitoring of the patients during chemotherapy will give an indication for the individualization of treatment schedules.

Germ-cell tumors have been classified mainly as seminomas and nonseminomas, with consequences for their treatment. In the near future, also other histological parameters (e.g. vascular and lymph vessel invasion) will be included in treatment strategies because of their prognostic importance (Sesterhenn et al). In a recently started investigation, these histological parameters will be included in the evaluation of treatment strategy in the patients. More experimental studies on cytogenics may aid decisions about those treatment schedules in the future.

Prostate cancer

Every patient with prostate cancer has to be treated on an individual basis. In the last fifty years, all patients with metastatic prostate cancer have been treated palliatively with hormones with no clear prolongation of survival. Lately, more and more attempts

have been made to develop new treatment options. In patients with low stage metastatic disease the question if early hormonal treatment gives a better survival and a longer time to progression than delayed therapy, is still under investigation. On the other hand, patients with extensive metastatic disease may profit from early combination of hormonal plus chemotherapeutic treatment. In a pilot trial of chemohormonal therapy for metastatic prostate carcinoma, this treatment showed good results and the study is now continuing in a large phase III study (Dawson et al.). The inclusion of patients in these studies are based on the results of prognostic factor analysis and are good examples for future strategy.

Careful monitoring of the response to treatment of patients with prostatic carcinoma has shown great value. In a recently started large study of patients with metastatic disease a search will be done for those patients whose reaction to hormonal treatment was less than expected from the use of prognostic factors. These patients may then be accesable for alternative options in order to improve their survival.

More basic research will yield additional prognostic factors in the near future, relevant with respect to the heterogeneity of the tumor and consequently the variability of success of hormone treatment. Sofar attempts to establish a functional relationship between the nuclear androgen receptor concentration and the duration of response to hormone therapy have been inconclusive. It is not inconceivable that more results will come from the detection of new monoclonal and polyclonal anti-androgen receptor antibodies. It is probable that the effect of hormonal treatment will be followed by measuring the androgen receptor content by occasionally performing biopsies at time intervals (Chodak et al).

One important aspect that we have learned from tumor cell biological evaluation is the fact that more aggressive tumors tend to be genetically unstable. This detection of heterogeneity in certain tumors has recently been seen in the multifocality with both diploid and nondiploid cancer cells present in the same prostate (Greene et al). Also the evaluation of tissue PSA heterogeneity in lymphnode metastasis can provide additional prognostic value (Hamdy et al). Molecular instabilities in prostate cancer are found in mutations in the P53 tumor suppressor gene and can stand for the likelihood of progression in these tumors (Effert et al). Also a decreased expression of the intercellular adhesion molecule E-cadherin showed biological significance in prostate cancer and may

give an indication as to the metastatic potential of the tumor (Umbas et al).

Bladder tumors

For superficial bladder tumors a more strict criteria for treatment continues to be of interest in the future. From the prognostic factor analysis from previous trials on treatment with adjuvant intravesical immunotherapy or chemotherapy we learned that not all the patients react identically to treatment. Much progress will be made in the research on 'case selection': which patient will benefit the most from which schedule of adjuvant intravesical therapy. Therefore, in the new trials on treatment which have been initiated recently, a more strict selection of patients has been adhered to. Also individualisation of follow-up schedules will be important.

In the near future the results showing proliferation antigens, growth factors, proteolytic enzymes and genetic alterations will exert their clinical impact (Lipponen et al). A good example here is the p53 antigen which has been implicated in the pathogenicity of bladder cancer (Sidransky et al). More studies are underway further to characterize these results and determine if other factors including ploidy, localized loss of heterozygosity and mutation at other tumor suppressor loci can be used in join to design a system of molecular prognostic markers for bladder cancer.

Renal cell carcinoma

For renal-cell carcinoma efforts will be made to clarify the still unpredictable clinical course of these patients. It is expected that in the near future we will be able to prognosticate more accurate by using cytometric, cytogenic and karyometric parameters. A good example is the proliferating cell nuclear antigen (PCNA) which can be used to identify patients with early disease which may have a poor prognosis (Cronin et al). The reproducibility of these new prognostic factors should still be confirmed. Every newly developed marker should be investigated in separate groups of patients in order to establish its importance for routine clinical practice. A good example is the nuclear morphometry which will be used for routine clinical practice with consequences for the management of patients with renal-cell carcinoma. The use is also advocated by several other investigations (Gutierrez et al). Next to the already existing staging systems, parameters like these will tell us more about the biological behaviour and the

aggressiveness of the tumor. The heterogeneous aspect of the tumor will have consequences for the follow up schedules and the suitability of adjuvant treatment.

Until now, the treatment of metastatic renal-cell carcinoma by immunotherapy has been characterized by the lack of response and severe side-effects. Progress will be made by a decrease of the side-effects of, especially, the immunotherapeutic agents. With the use of a prognostic factors analysis, it is becoming more and more clear that selected patients react better to this kind of treatment than a random group (de Mulder). With these two issues in mind, new trials will therefore be developed to produce a better response to these kinds of treatment.

In a recently initiated study, we include the value of the karyometric analysis in a karyometric malignancy index. With the results of this method, the prognosis of the individual patient may be predicted with great accuracy. In the near future, further individualization of the management of patients with renal-cell carcinoma will be possible. This may lead to an improvement in survival of those patients.

The search for prognostic factors in urological tumors will be continuous. The results from this thesis must therefore be at the basis for further research. Every analysis on prognostic factors will initiate new investigations in this field. Essential towards this goal is a good interaction between basic research and clinical investigations. Success in basic research must not lead to complacency in clinical studies and vice versa. Only in this collaborative way the best possible prognostic parameters will be found. In the end, this is the only way which will lead to the improvement in the indication and the choice of treatment and therefore the improvement of the survival of patients with urological oncological disease.

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HOOFDSTUK X

SAMENVATTING EN TOEKOMSTPERSPECTIEVEN

1. Samenvatting

In **Hoofdstuk I.1** wordt de gedachtengang van het onderzoek naar prognostische factoren in de oncologische urologie besproken. Geconcludeerd wordt dat het mogelijk is de exacte waarde van de diverse prognostische factoren te bepalen en dat deze 'prognostische stratificatie' als basis kan dienen voor het ontwerpen van nieuwe behandelingsprotocollen. In deze context is de bijdrage van de statisticus van essentieel belang, mede vanwege het feit dat de keuze van de methode van de statistische analyse op een zo objectief mogelijke manier dient te geschieden. In **Hoofdstuk I.2** wordt een overzicht gegeven van de tot nu toe bekende prognostische factoren bij de tumoren van de testis Verschillende histologische classificatie- en stagieringsmodellen worden beschreven en hun belang ten aanzien van de keuze van de behandeling en de prognose wordt benadrukt. Een combinatie van stagierings, histologische en biochemische parameters maakt het mogelijk risico-groepen te definieren. Deze dienen dan als basis voor verder onderzoek naar prognostische factoren in deze tumoren In **Hoofdstuk I.3** wordt een samenvatting gegeven van de belangrijkste prognostische factoren bij het gemetastaseerde prostaatcarcinoom. Met name de biochemische parameters zijn van belang voor het bepalen van de prognose en het vervolgen van de ziekte. Deze factoren zijn, naast de histologische en radiografische parameters, van invloed op de prognose van de individuele patient In **Hoofdstuk I.4.** worden de prognostische factoren van het oppervlakkig blaascarcinoom besproken. Met behulp van de klinische en pathologische parameters is het mogelijk de kans op een recidief carcinoom gedurende de follow-up te voorspellen Daar steeds meer verschillende behandelingen worden ontwikkeld, is de waarde van deze prognostische parameters van toenemend belang. In **Hoofdstuk I.5** wordt een samenvatting gegeven van de prognostische factoren bij tumoren van de nier. Ondanks de aanwezigheid van de reeds bekende klinische, pathologische, en biochemische prognostische parameters, blijft het moeilijk het beloop van de ziekte exact te voorspellen. De prognostische factoren die van invloed zijn op de prognose worden in dit hoofdstuk besproken. De recente vooruitgang in het detecteren van cytogenetische en karyometrische karakteristieken van de tumorcellen worden eveneens belicht.

Het resultaat van het overzicht van de prognostische factoren zoals dat gegeven is in hoofdstuk I vormt het uitgangspunt voor de onderzoeken die verricht zijn in dit

proefschrift. Met de reeds bekende prognostische factoren als basis, zijn nieuwe onderzoeken geïnitieerd met als doel de prognose van een patient met een urologische tumor beter te kunnen definiëren. Met de resultaten hiervan kunnen adviezen verkregen worden voor het instellen van de juiste behandeling en het juiste follow-up schema.

In **Hoofdstuk II** onderzoeken we de waarde van de reeds bekende classificatie modellen van de "European Organisation for Research and Treatment of Cancer (EORTC)" en de Indiana (VS) groep, met betrekking tot de patiënten in het Radboudziekenhuis met een gemetastaseerde testis tumor. Beide modellen waren bruikbaar ten aanzien van het voorspellen van de overleving van deze patiënten die primair chemotherapeutisch werden behandeld. De grootte van de retroperitoneale tumor voor en na de chemotherapie bleek hierbij van additionele prognostische waarde. De radiografische verandering in de grootte van de retroperitoneale tumor gedurende de chemotherapeutische behandeling bleek een voorspellende waarde te hebben voor de histologie van het weefsel dat na de chemotherapie gereseceerd werd. Dientengevolge was deze bevinding van invloed op de prognose. Dit resultaat maakt een verdere individualisering van de behandeling mogelijk.

In **Hoofdstuk III** wordt een update gegeven van prognostische factoren bij het gemetastaseerd prostaatcarcinoom. Deze parallel studie van een tweetal goed gedocumenteerde trials van hormonale behandelingen, toont aan dat met behulp van de parameters: algemene conditie, hemoglobine-gehalte en de hoogte van de alkalische fosfatase in het serum, bepaalde risicogroepen kunnen worden gevormd. Met name voor de, op deze wijze geformeerde groep met een slechte prognose, wordt geadviseerd alternatieve vormen van behandeling te onderzoeken om daarmee de prognose te verbeteren.

In **Hoofdstuk IV** wordt de exacte waarde van de biochemische markers: alkalische fosfatase, prostaat zure fosfatase en het prostaat specifieke antigeen (PSA) onderzocht bij patiënten met een gemetastaseerd prostaatcarcinoom. Dit met name ten aanzien van het bepalen van de reactie van het carcinoom op de hormonale medicatie. Vooral het PSA gehalte bleek hierbij van waarde en kan zelfs het routinematig controleren van de bot-scintigrafie overbodig maken. Van deze procedure van monitoring wordt inmiddels gebruik gemaakt in de laatste trials.

In **Hoofdstuk V** wordt een controversiële subgroep van patiënten met een

oppervlakkig blaascarcinoom besproken. De resultaten van de behandeling van patiënten met pT_1G_3 blaastumoren, verzameld door de urologen van het zuid-oost nederlands samenwerkingsverband, werden geëvalueerd, gebruikmakend van een retrospectieve techniek. Geen van de onderzochte parameters bleek additionele prognostische waarde te hebben binnen deze, door tumorstadium en gradering geselecteerde en daardoor homogene, groep. Vanuit deze studie kon ook geconcludeerd worden dat, na de transurethrale resectie van de tumor, aanvullende behandeling door middel van intravesicale immuno-, chemotherapie danwel radiotherapie vereist is om het recidief-vrije-interval te verlengen.

In **Hoofdstuk VI** wordt een studie verricht naar prognostische factoren bij patiënten met een oppervlakkig blaascarcinoom, die allen nabehandeld werden met intravesicale installaties van immuno- danwel chemotherapie. In deze goed gedocumenteerde trial bleken het aantal tumoren en de lokalisatie van de tumor in de blaas van prognostische waarde te zijn voor het recidief-vrije-interval. Vooral het voorkomen van minstens één tumor in het trigonum danwel in de achterwand van de blaas bleek een grotere kans te geven op een tumor recidief gedurende de follow-up. Geconcludeerd werd dat met name deze at risk patiënten een stringente follow-up behoeven.

In **Hoofdstuk VII** onderzoeken we door middel van een prognostische factor-analyse de waarde van de klinische, flowcytometrische en karyometrische karakteristieken bij patiënten met niertumoren. In deze heterogene groep van patiënten met alle stadia van het niercarcinoom, was het duidelijk dat van de klinische factoren alleen de algemene conditie van de patient en het gewichtsverlies van invloed waren op de prognose. Van de karyometrische parameters waren de karakteristieken die de heterogeniteit binnen de tumor uitdrukken, van belang voor de prognose van het niercelcarcinoom. Deze heterogeniteit zou kunnen staan voor de biologische onstabiliteit van de tumor en daarmede dus de overleving verslechteren. De flowcytometrische parameters vertoonden geen aanvullende prognostische significantie, maar dit zou veroorzaakt kunne zijn door het feit dat slechts één tumorsample werd onderzocht. De bovengenoemde heterogeniteit was dus niet beschikbaar voor de analyse.

In **Hoofdstuk VIII** wordt een subgroep van $T_{1-3}N_0M_0$ niertumoren onderzocht ten aanzien van prognostische factoren. Deze studie benadrukte de waarde van de

karyometrische parameters; die parameters die staan voor de heterogeniteit van de tumor blijken van het grootste belang te zijn. In deze context geeft karyometrie informatie over de maligniteitspotentiaal van de tumor en kan derhalve gebruikt worden voor het selecteren van de juiste behandeling, bijvoorbeeld de immunotherapie.

2. Toekomstverwachtingen

De studies betreffende de **analyse van prognostische factoren in urologische tumoren** zullen in de toekomst een belangrijke rol blijven spelen. Naarmate het aantal behandelingsmogelijkheden toeneemt, zullen passende prognostische factoren nodig zijn voor de evaluatie. Deze prognostische stratificatie zal verder bijdragen tot een verbetering van de behandelingsresultaten bij patiënten met een carcinoom. Iedere fase III gerandomiseerde studie zal begeleid dienen te worden door een zogenaamde side-studie van prognostische factoren. Deze goed gedocumenteerde studies geven ons de gelegenheid om de exacte waarde van de verschillende prognostische factoren te bepalen bij relatief homogene groepen van patiënten. Om het mogelijk te maken relevante conclusies te trekken is het nodig om een respectabel aantal patiënten te verzamelen binnen een korte tijdsperiode. Het is daarom van belang te adviseren grote internationale trials op te zetten. Dit onderstreept het belang van internationale organisaties als de European Organisation for Research and Treatment of Cancer (EORTC). De waarde van de prognostische factor analyse wordt ook verhoogd door de bestudering van een groot aantal patiënten, die met gebruikmaking van dezelfde inclusie en exclusie criteria worden verzameld, en die behandeld worden binnen een relatief korte tijdsperiode. Alleen op deze manier kan een nauwkeurige evaluatie van de behandeling van de individuele patient worden gegarandeerd.

Om vooruitgang te boeken in de oncologische urologie is het noodzakelijk het onderzoek naar prognostische factoren voort te zetten. De waarde van de prognostische factoren moet worden gedefinieerd voor de verschillende stadia van de tumor en de betreffende patient. Ten aanzien van het fundamentele onderzoek, dienen de cytometrische, cytogenetische en moleculaire afwijkingen uitgebreid onderzocht te worden, zodat deze in de toekomst gebruikt kunnen worden in de routine klinische setting.

Testis tumoren

Verder onderzoek naar het juiste tijdstip en de intensiteit van de verschillende behandelingsfasen van de testis tumoren zal gedaan moeten worden. Nieuwe analyses van prognostische factoren van grote groepen patiënten, zullen uiteindelijk beter de verschillende risicogroepen kunnen definiëren (Mead e.a.). Vooral die patiënten die het risico lopen niet goed op de bestaande behandeling te reageren zullen moeten worden opgenomen in nieuwe experimentele studies. Daar deze zeer intensieve chemotherapeutische behandelings schema's nog steeds ernstige en in potentie letale bijwerkingen kunnen hebben, is het identificeren van deze patiënten met behulp van een prognostische factor analyse van het grootste belang.

Vanwege de zeldzaamheid van de tumor is het van belang dat groots opgezette studies met medewerking van meerdere centra worden ontwikkeld, met als doel de reeds relatief goede prognose verder te verbeteren. Vooral meer individualisatie van de behandeling is gewenst; b.v. het bepalen van het juiste aantal chemotherapie kuren, danwel de juiste indicatiestelling tot lymfklier chirurgie. Tevens zal een betere registratie van de reactie van de tumor op chemotherapie een indicatie geven voor het verder individualiseren van de behandelingsschema's.

Testis-tumoren worden tot nu toe vooral ingedeeld in seminoma's en non-seminoma's, met directe consequenties voor de behandelingsmethode. In de nabije toekomst zullen ook andere histologische parameters van de primaire tumor (b.v. vaatinvasie en lymfklierinvasie) worden meegenomen in de beslissing ten aanzien van de behandelingsstrategieën (Sesterhenn e.a.). In een studie die zojuist gestart is, worden deze histologische parameters betrokken bij de evaluatie van de behandeling van deze patiënten. Meer experimentele studies naar cytogenische parameters zullen misschien van nut zijn voor het bepalen van de behandeling in de nabije toekomst.

Prostaatcarcinoom

Iedere patient met een prostaatcarcinoom zal op individuele basis moeten worden behandeld. De laatste 50 jaar zijn alle patiënten met een gemetastaseerd prostaatcarcinoom palliatief behandeld met hormonale medicatie, zonder dat daardoor de overlevingsduur evident werd verlengd. De laatste tijd worden steeds meer pogingen gedaan om nieuwe behandelingsmogelijkheden te ontwikkelen. Bij patiënten met een

prostaatcarcinoom met een geringe metastasering, wordt onderzocht of directe hormonale behandeling een betere prognose geeft dan alleen dan behandelen wanneer er klachten zijn. Aan de andere kant wordt onderzocht of patiënten die uitgebreid gemetastaseerd zijn beter behandeld kunnen worden door middel van het direct starten met een combinatie van hormonale- en chemotherapie. Een pilot-studie van deze chemo-hormonale behandeling voor het gemetastaseerde prostaatcarcinoom vertoonde veelbelovende resultaten, en deze studie wordt op dit moment gecontinueerd in een grote fase III studie (Dawson e.a.). De selectie van patiënten voor deze studies is onder andere gebaseerd op de resultaten van de prognostische factor analyses uit het verleden en zijn goede voorbeelden voor de toekomstige behandelingsstrategie.

Een zorgvuldige registratie van de reactie van het prostaatcarcinoom op de ingestelde hormonale behandeling is van groot belang gebleken. In een recent gestart onderzoek wordt getracht die patiënten te detecteren die minder goed reageren op de hormonale behandeling dan men zou verwachten op basis van de prognostische factor analyses. Deze patiënten zouden dan in aanmerking moeten komen voor die alternatieve behandelingsmethoden, die de overlevingsduur verbeteren.

Uitgebreider experimenteel onderzoek zal in de nabije toekomst nieuwe prognostische parameters opleveren die de heterogeniteit binnen de tumor aangeven, met als consequentie een uiteenlopende reactie op de hormonale behandeling. Tot nu toe zijn de pogingen om een duidelijke relatie aan te tonen tussen androgeen receptor concentratie van de celkern en de duur van de respons op de hormonale behandeling, niet conclusief. Het is niet ondenkbaar dat meer resultaten zullen komen betreffende de detectie van nieuwe monoclonale en polyclonale anti-androgeen receptor anti-lichamen. Tevens is het mogelijk dat, door middel van het nemen van prostaatbiopsieën op bepaalde tijdstippen gedurende de follow-up, het effect van de hormonale behandeling gemeten zal gaan worden (Chodak e.a.).

Een belangrijke bevinding vanuit de tumorcel biologie, is het feit dat meer agressieve tumoren de neiging hebben genetisch onstabiel te zijn. De ontdekking van de heterogeniteit in bepaalde tumoren is recentelijk tot uitdrukking gekomen in de multifocaliteit van zowel diploïde als non-diploïde cellen, aanwezig in dezelfde prostaat (Greene et al). Ook de weefsel-PSA concentraties kunnen verschillend zijn binnen dezelfde tumor, waardoor deze heterogeniteit in, met name de lymfkliermetastasen

additionele informatie zou kunnen geven ten aanzien van de prognose (Hamdy e.a.). Moleculaire instabiliteiten bij het prostaatcarcinoom worden gevonden bij het P53 tumor suppressie gen en kunnen een indicatie geven voor de kans op progressie (Effert e.a.). Ook een verminderde expressie van het intercellulaire adhesie molecuul E-cadherin toont een biologische significantie ten aanzien van de prognose van het prostaatcarcinoom door middel van zijn invloed op de kans op het ontwikkelen van tumor metastasen (Umbas e.a.).

Blaastumoren

Een strikte indicatiestelling voor het starten van de juiste behandeling voor het oppervlakkig blaascarcinoom, blijft van belang in de nabije toekomst. Van de prognostische factor analyses die gedaan werden bij voorgaande behandelingen met adjuvante intravesicale immuno- en chemotherapie, weten we dat niet iedere tumor op dezelfde manier reageert op deze behandeling. Veel vooruitgang zal worden geboekt in de juiste selectie van patiënten voor de juiste behandelingsstrategie. Derhalve zal in de nieuw te ontwikkelen trials, een meer strikte indicatiestelling worden toegepast. Tevens zal een individualisatie van de follow-up schema's worden nagestreefd.

In de nabije toekomst zullen de resultaten aangaande proliferatie antigenen, groeifactoren, proteolytische enzymen en genetische alternaties hun klinische toepassing bewijzen (Lipponen e.a.). Een goed voorbeeld is het p53 antigeen dat van belang blijkt te zijn voor de pathogeniciteit van het blaascarcinoom (Sidransky e.a.). Meer studies zijn in aantocht om de waarde van deze bevindingen al dan niet te bevestigen en zullen dan vervolgens bepalen of andere factoren zoals ploïdiciteit, verlies aan heterogeniteit en mutaties op andere tumor suppressor lokaties kunnen worden opgenomen in een systeem van moleculaire prognostische markers voor het blaascarcinoom.

Nierceltumoren

Ten aanzien van deze tumoren is het van belang dat verder onderzoek naar het, nog steeds onvoorspelbare klinische beloop van deze patiënten verricht dient te worden. Verwacht kan worden dat in de nabije toekomst een meer accurate voorspelling zal worden gedaan ten aanzien van de prognose van deze tumoren, gebruikmakend van de cytometrische, cytogenetische en karyometrische parameters. Een goed voorbeeld is het

prolifererende celkern antigeen (PCNA), dat gebruikt kan worden om patiënten te identificeren die kleine carcinomen hebben met een slechte prognose (Cronin e.a.). De reproduceerbaarheid van deze nieuwe prognostische factoren moet echter nog worden onderzocht. Iedere nieuw ontdekte marker moet worden onderzocht binnen verschillende groepen patiënten om op deze manier het belang voor de dagelijkse klinische praktijk te bewijzen. Een goed voorbeeld hiervan is de morfometrie van de kern. Deze zal gebruikt gaan worden in de routine klinische praktijk en uiteindelijk consequenties hebben voor de behandeling van patiënten met niercel carcinomen. Het gebruik van de karyometrie voor het bepalen van de prognose wordt ook aangeraden door andere onderzoekers (Gutierrez e.a.). Naast de reeds bestaande stagiëringssystemen zullen deze parameters informatie geven over het biologische gedrag en de agressiviteit van de tumor. De mate van heterogeniteit van de tumor zal consequenties hebben voor de follow-up schema's en een indicatie geven voor een eventuele adjuvante behandeling.

Tot nu toe wordt het gebruik van adjuvante immunotherapie bij patiënten met een gemetastaseerd niercelcarcinoom gekarakteriseerd door een gebrek aan respons en het voorkomen van relatief ernstige bijwerkingen. Vooruitgang zal worden geboekt in het verminderen van deze bijwerkingen van vooral de immunotherapeutische middelen. Gebruikmakend van de resultaten van de prognostische factor analyses wordt het steeds duidelijker dat voor geselecteerde patiënten de reactie op deze behandelingsmethoden gunstiger is dan voor de gehele groep (de Mulder e.a.). Met deze twee uitgangspunten in het achterhoofd zullen nieuwe trials worden ontwikkeld, met als doel een betere respons van de niertumor op de behandeling te verkrijgen.

In een recent gestarte trial maken we gebruik van de resultaten van de karyometrische analyses, uitgedrukt in de karyometrische maligniteits index. Door middel van deze methode kan de prognose van de individuele patient met een grotere nauwkeurigheid bepaald worden. In de nabije toekomst zal verdere individualisatie van de behandeling van patiënten met niertumoren mogelijk worden gemaakt. Dit zal dan uiteindelijk leiden tot een verbetering van de overleving van deze patiënten.

Onderzoek naar prognostische factoren is een continu proces. De resultaten van dit proefschrift moeten derhalve aan de basis staan van toekomstig onderzoek. Iedere prognostische factor analyse zal nieuwe onderzoeken op zijn terrein initiëren. Essentieel

hierbij is het combineren van de resultaten van experimenteel en klinisch onderzoek bij patiënten met kanker. Het succes in het experimenteel wetenschappelijk onderzoek moet niet leiden tot een vermindering van het klinische onderzoek en vice versa. Alleen door goede samenwerking zullen de best mogelijke prognostische parameters worden gevonden. Uiteindelijk is dit de enige manier een verbetering te verkrijgen in de indicatiestelling voor de juiste behandeling teneinde de overleving te verbeteren voor patiënten met een urologische oncologische ziekte.

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CURRICULUM VITAE

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De schrijver van dit proefschrift werd geboren op 31 mei 1962 te Huissen. Hij behaalde in 1980 het diploma voor het voorbereidend wetenschappelijk onderwijs (V.W.O.) aan het Thomas à Kempis College te Arnhem.

Na enkele maanden gestudeerd te hebben aan de Landbouw Hogeschool te Wageningen werd eind 1980 aangevangen met de studie Geneeskunde aan de Katholieke Universiteit Nijmegen. Het doctoraalexamen werd afgelegd in 1985. Van 1 mei tot 1 september 1987 werkte hij als student-doctor in het Agogo Hospital te Ghana (West-Afrika). Het artsexamen werd afgelegd in januari 1988.

Na 1 maart 1988 voldeed hij gedurende 16 maanden de militaire dienstplicht als officier-arts, gelegerd te Hilversum, Schaarsbergen en Nijmegen.

Van 1 juli 1989 tot 1 januari 1992 was hij voor de B-opleiding algemene heelkunde werkzaam in het Ikazia ziekenhuis te Rotterdam (opleiders: Dr. A.P. Brinkhorst, Dr. H.F. Veen). Vanaf 1 januari 1992 is hij als arts-assistent in het kader van de opleiding tot uroloog verbonden aan de afdeling Urologie van het Sint Radboudziekenhuis (Opleiders: Prof. Dr. F.M.J. Debruyne, Dr. G.O.N. Oosterhof).

Vanaf 1989 werd naast de opleiding het onderzoek verricht dat tot deze dissertatie heeft geleid.

Hij is gehuwd met Cindy Hugen, kinderarts in opleiding.

Stellingen behorende bij het proefschrift

Prognostic Factors

in

Urological Tumors

door

P.F.A. Mulders

Nijmegen, 4 mei 1993

1. Monitoring van het gemetastaseerd testiscarcinoom gedurende de chemotherapie is van prognostische belang en kan leiden tot individualisatie van de behandeling. (*dit proefschrift*)

2. Bij patiënten met een gemetastaseerd prostaatcarcinoom is het mogelijk met behulp van prognostische factoren risico-groepen te vormen, welke consequenties kunnen hebben ten aanzien van de behandeling. (*dit proefschrift*)

3. Het Prostaat Specifiek Antigeen (PSA) is een adequate parameter voor de monitoring van behandeling van patiënten met een prostaatcarcinoom. (*dit proefschrift*)

4. T1G3 blaascarcinomen vormen een homogene groep en dienen na transurethrale resectie adjuvant behandeld te worden om het recidief-vrije interval te verlengen. (*dit proefschrift*)

5. Multipliciteit en locatie in de blaas van de met intravesicale instillaties behandelde oppervlakkige blaastumoren zijn van prognostische waarde voor het recidief-vrije interval. (*dit proefschrift*)

6. Karyometrische parameters hebben bij het niercelcarcinoom additionele prognostische waarde, en kunnen derhalve van invloed zijn op de keuze van de behandeling. (*dit proefschrift*)

7. Antenatale interventie bij nefro-urologische afwijkingen dient met de grootste terughoudendheid te worden benaderd, daarentegen kan de antenatale echoscopie een belangrijke bijdrage leveren aan de preventie van nierbeschadiging.
C.A.C. Hugen (Ned Tijdschr Geneeskd 1989; 133,nr 34)

8. Patiënten met een vasectomie in de voorgeschiedenis hebben een verhoogde kans op het krijgen van prostaatcarcinoom (*E. Giovannucci, JAMA 1993, 873,269*); de reden hiervan dient echter aanvullend onderzocht te worden, en zou kunnen leiden tot een beter begrip van de etiologie van het prostaatcarcinoom.

9. De chirurgische benadering blijft voor patiënten met een urologische tumor de enige kans op curatie, en moet derhalve, indien mogelijk, centraal staan bij iedere behandeling.

10. Vroege detectie van een kwaadaardig gezwel kan levensreddend zijn, derhalve dienen kosten nog moeite te worden bespaart om dit te bewerkstelligen.

11. Het doen van basaal wetenschappelijk onderzoek moet niet in de plaats komen van het klinisch onderzoek, maar dit aanvullen om de klinische relevantie te waarborgen.

12. Door de medicus practicus dient het woord "significant" met de nodige statistische argwaan te worden geïnterpreteerd.

13. Een slecht chirurg is een chirurg die de kwaliteit van zijn operatie volledig relateert aan de snelheid waarmee de operatie wordt verricht. *Dr. H.F. Veen, chirurg*

14. De computer is een onmisbaar werktuig geworden voor de moderne promovendus; het betekent echter ook dat deze de meeste secretariële ondersteuning kan, maar ook moet ontberen.

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